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Vol. III. *May and June, 1902.* *Nos. 1 and 2.*

BIOLOGICAL BULLETIN.

MATURATION OF THE EGGS OF DIEMYCTILUS TOROSUS.

DR. HECTOR LEBRUN, BRUSSELS, BELGIUM.

During my stay in California, where the Belgian government sent me to verify the results of Dr. Eisen's investigations on the testicles of the Batracoceps, and to study the eggs of these and other Batrachians, the occasion also offered itself to study the maturation process in *Diemyctilus torosus.*

I knew, by the work of Professor Ritter, of the University of California, that the copulation of this species takes place during the months of March and April, and therefore immediately devoted my investigations to this subject. My material came from Tocoloma, Marin County, California, where Professor Van der Naillen called my attention to a large quantity of specimens. In my previous publications, I have shown it to be absolutely necessary to have the largest possible number of specimens on hand to have good material in the different stages of the maturation of the eggs, because these phenomena last only a few days, and during this period, it is necessary to kill a large quantity of females. All of these conditions are perfectly realized in Tocoloma.

By the kindness of the San Francisco Microscopical Society, which generously opened to me its rooms and instruments, I began to study the material collected, and demonstrated my preparations at a meeting of the Society on the 15th of May.

As the present note is preliminary and a summary of the researches made in California, my investigations will be continued in Europe and published in the review (La Cellule).

The ovary of *Diemyctilus* contains from five to six species of eggs in different stages of development. I will not attempt to detail the history of these eggs from ovogony to the maturation stage, but will only state that, as a result of my investigations, I have found absolute confirmation of my researches on European urodels and on Siredon.

I will here limit my references to the process of the maturation of the egg.

When the egg is ripe, the nucleus is situated at the animal pole, voluminous, and contains four to five hundred chromatic nucleoles, in various stages of dissolution, and resolving into granula. The first appearance of maturation is the vacuolization of the karyoplasma and the dissolution of all the granular chromatin, except a little quantity of nucleoles. These have in this stage a propensity to fuse together. Shortly, the membrane of the nucleus disappears, and during this stage, the chromatic element is represented in the egg by spherical nucleoles and by masses made by fusion of other nucleoles.

All these phenomena are finished in the ovaries. When the egg is found in the peritoneum the spindle of the first polar body is finished and the chromosomes fixed on the spindle. These are formed in a small region of karyoplasma which is not invaded by the cytoplasmic inclusions after the disappearance of the nuclear membrane. There are no centrosomes, but beautiful asters. The filaments of these orders are crossed on the equatorial plane of the figure as is known, by *Batrachoceps* (Eisen), by pollen-mutter cells (Osterhout and his pupils) and tritons (Lebrun). The first figure and the expelling of the first polar body is finished in the superior part of the oviduct. The second figure runs out during the egg's passage through the oviduct to the cloaca. The chromosomes are twelve as in the other urodels. They are quadripartite as in the first polar figure and have the form of a cross, resembling those found in European tritons and called "oiselets." I will not, by this, say that I give to these forms the same signification as do Von Rath, Haecker, and others. On the contrary, I conclude that in the two figures the chromosomes divide longitudinally in the equatorial plane.

SAN FRANCISCO, CAL., May 15, 1901.

NEW SPECIES OF CERATOPOGON.[1]

WILLIAM HENRY LONG, JR.

The family Chironomidæ has been much neglected by dipterologists, notwithstanding the number and diversity of its species. Even the larval forms which are especially interesting have not, as yet, received as much study as they deserve. The writer's attention was first directed to this family by finding the larvæ of a species of *Ceratopogon* in large numbers in the vicinity of Austin, Texas, during the winter months. Certain peculiarities of its development induced him to extend his study to other species of *Ceratopogon* in the same vicinity. This study brought to light several interesting facts, some of which may prove of value in separating the species of this large and complex genus. Two of the species included in this article seem to be myrmecophilous, the first, it seems, to be recorded from America.

CERATOPOGON BRUMALIS, sp. nov.

♂, length 3 mm., wing 2 x .5 mm.; ♀, length 2.5 mm., wing 2 x .75 mm. Two subcostal cells; third or cubital vein terminating distinctly before the middle of the wing with a white spot at apex of costal vein; wings and entire insect densely pilose; halteres white; metatarsi equaling or slightly shorter than the following joint: tibia of female with black lanceolate scales; last four joints of the male's antennæ longer than the basal ten joints; eleventh about 1.5 times as long as any succeeding joint. Antennæ not longer than mesonotum.

♀, head piceous; pile on tips of antennæ and palpi whitish; joints 2–9 of antennæ ovate subequal, joint 1 much larger and more globose; joints 10 to 14 inclusive ovate-oblong; apical joint prolonged into a blunt point. Each of the joints 2–9 inclusive has two large sense organs, which are curved, transparent and on upper surface of joint about 90° apart; other and much smaller sense organs occur near the apex, three or more to each joint; while joints 13 and 14 are fairly covered with minute organs of similar nature. Each joint, except the first, is pubescent and bears a circlet of bristles. Mesonotum dark brown, pruinose, pile brassy; sides of prothorax with a bunch of yellow bristles; pleuræ naked; mesopleuræ white; abdomen black, shining, pile light yellow, reddish in certain lights; venter brown; external genitalia subglobose,

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 17.

yellowish white ; legs yellowish brown, strongly infuscated ; knees yellow ; tibiæ spurred, outer edge with a row of black lanceolate scales ; bases of tarsi light yellow ; first two joints subequal, the others shorter ; each joint with a graduated row of short bristles on the plantar surface ; empodium strap-shaped, bristly-fringed, nearly as long as claws, claws subequal. Neuration of wings [1] as follows : Costal vein strong, terminating distinctly before the middle of the wing, pilose except for a small naked spot near the base ; third or cubital vein strong, terminating at apex of costal vein, with

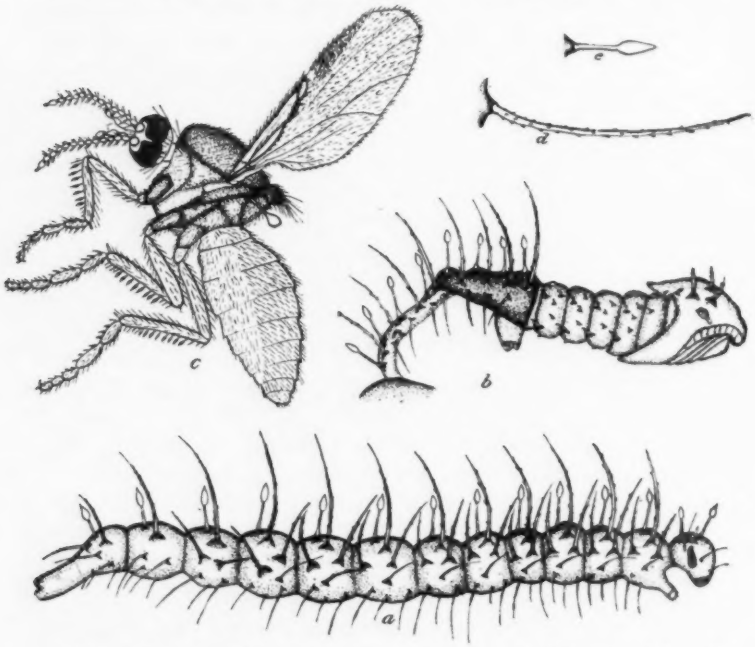


FIG. 1. *Ceratopogon brumalis*. *a*, larva ; *b*, pupa ; *c*, female imago ; *d*, lateral barbellate bristle ; *e*, dorsal ovate-lanceolate bristle.

a strong auxiliary vein extending from upper end of recurrent vein to juncture of costal and cubital veins ; nearly midway between the upper end of recurrent vein and apex of cubital, the latter vein curves forward abruptly till it touches the auxiliary vein, making two loop-like subcostal cells ; the second of which is much the larger ; subcostal vein a mere point of juncture ; recurrent vein strong, making a very obtuse angle with base of median vein, latter strong for the first third of its length, then distinct, but delicate to to margin of wing. Discal vein obsolescent before its juncture with the

[1] Nomenclature of neuration of wings after J. Winnertz.

median vein so that no prefurca is visible ; axillary vein distinct from its base to apex terminating in the posterior margin of the wing ; posterior vein short but distinct ; anal veins more or less indistinct, especially near base ; Anterior costal cell long and narrow with apex acute ; subcostal cells loop-like, first very small, often wanting, represented by the line of fusion of the auxiliary and cubital veins ; anterior cubital cell narrow, but enlarged at apex ; second cubital cell with two obsolescent veins which join near base of cell, making a V shaped figure ; between the upper anterior one of these veins and the margin of the wing, the cubital cell is densely pilose, except the small white spot at the juncture of the costal and cubital veins produced

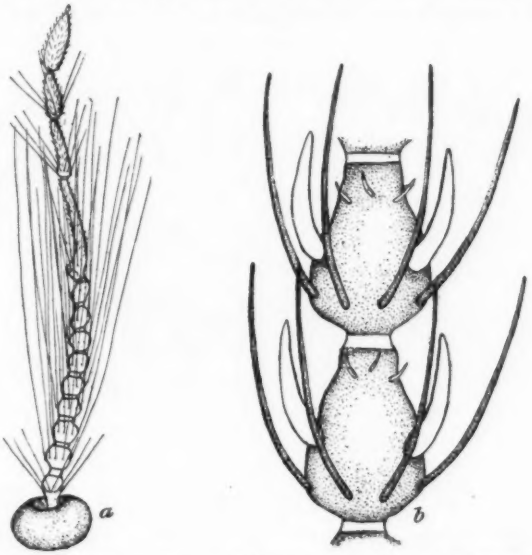


FIG. 2. *Ceratopogon brumalis*. *a*, male antenna ; *b*, two joints of female antenna showing sense organs.

by the absence of pile and by the hairs on the contiguous veins being whitish at that point. Two discal cells, subequal with an obsolescent vein in the posterior part of the second parallel with the auxiliary vein.

Coloration of male nearly the same as that of the female : differs from female as follows : Pile on entire insect longer, denser and of a more reddish hue ; the tibiæ without the lanceolate scales, metatarsi slightly but distinctly shorter than following joint ; abdomen slender, very pilose, black, slightly longer than wings. The most prominent divergence from female is in the shape and size of the joints of the antennæ ; first joint is much larger than the corresponding joint of the female ; the second somewhat larger but of the same shape, each joint from the third to the tenth spheroidal, becoming

more and more distorted and oblique to the tenth, which is of an irregular oblong shape. Joint 11 is much elongated with the plumosity inserted on an oblique line near base; joints 12–14 much shorter than 11, but longer than any of the basal ten joints. Joints 2, 12 and 13 with a circlet of bristles in place of plumosity; joint 1 naked, 14 without bristles or plumosity but pilose, as are joints 11, 12, and 13 in addition to the plumosity and bristles; joints 3–11 inclusive plumose with a narrow white circlet in front of the insertion of plumosity. Antennary sense organs smaller and straighter than those of the female. Neuration of the male wing practically as in female except that the first subcostal cell is wanting; its place being occupied by the fusion line of the auxiliary and cubital veins; anal veins stronger and more distinct than in female.

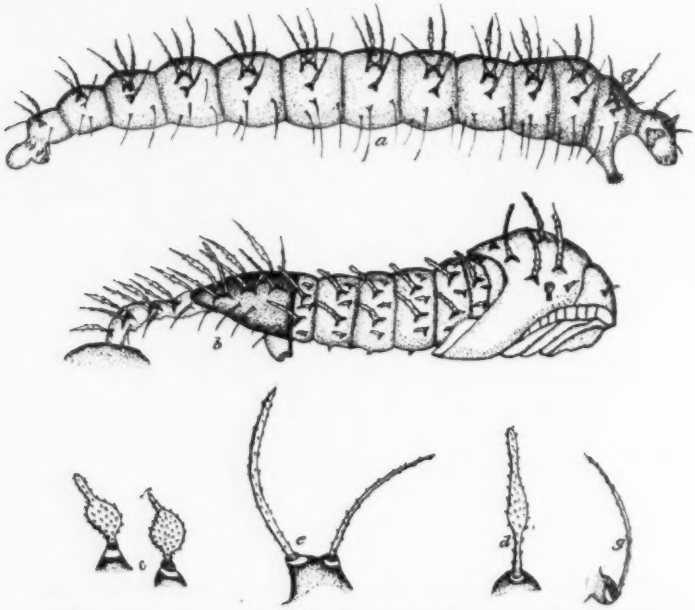


FIG. 3. *C. specularis.* *a*, larva; *b*, pupa; *c*, pair of prothoracic bristles; *d*, dorsal bristle on remaining segments; *e*, furcate tubercle with its two bristles; *g*, small lateral bristle.

Larva: 4–5 mm. long, cylindrical, color dirty white, brownish at the insertion of bristles; head brown, eye spot and mouth parts black. Thoracic legs, .3 mm. long, cylindrical, white, armed at tips with a circlet of black claws; anal feet with two transverse rows of black claws. Chætotaxy for lateral half of head and body as follows: Head with two ovate-lanceolate hyaline bristles, one in front of, the other behind the

typical head-spine or horn, in addition to several scattered simple bristles. Body minutely pubescent; ovate lanceolate bristles in one dorsal longitudinal row; thoracic pair not enlarged; dorsal bristles smooth, hyaline, bulbous at base. The insertion of the pair on each segment often connected by a transverse black line; 3 longitudinal lateral rows of barbellate bristles inserted below the ovate-lanceolate ones; bristles black, shining, with short and smooth bristles near the ventral surface, the last two segments having only the upper row of these barbellate bristles.

Pupa: 3 mm. long, spinous, light yellow; head darker, obtuse, notched below; thorax with three spines on either side, spines barbellate, anterior one mucronate; stigma spatulate; abdominal spines short, rough, mucronate, in five longitudinal rows on either side; those of rows 1 and 4 larger and longer than the others; there is also a ventral row of very short thick spines. Pupa attached to under surface of dung by the old larval skin.

During November, December and January the larvæ of this species were found in immense numbers on the under side of nearly dry cow dung. They seem to feed on the dung, never penetrating very far into the substance. No eggs were found. The duration of the larval stage seems to be several weeks, that o the pupal stage 7–10 days.

The sense organs on the antennæ of the imagines can be seen only with a high power (500 dia.) and then it is necessary to focus carefully to find them, but once found, they stand out plainly. None of the wings of the males had two subcostal cells and many wings of the females had only one; but in some wings the two cells show plainly. Several hundred larvæ of all ages were found on the under surface of a piece of moist rotting elm wood; similar larvæ and puparia were also found in the nests of the common foraging ant (*Eciton cæcum*) on several different occasions; the author bred imagines from the larvæ taken in these various habitats, and they proved to be the same species. This seems to be a strictly winter species, none of the various stages being found during the warmer months.

CERATOPOGON SPECULARIS Coquillett.

♂, length 2.5 mm., wing 1.5 x .45 mm.; ♀, length 2 mm., wing 1.5 x .6 mm. One subcostal cell, third or cubital vein terminating distinctly before the middle of the wing; with a faint white spot at apex of costa vein; wings and entire insect densely pilose; pile black; halteres white; metatarsi equalling or slightly longer than following joint; antennæ not

longer than mesonotum ; tibiæ of the female without lanceolate scales ; last four joints of male antennæ longer than basal ten.

♀, head black, both extremities of each segment of the palpi whitish, especially the anterior extremities ; central and major portion of each segment black ; proboscis with three narrow transverse whitish bands near apex. Pile on antennæ black, whitish at tips ; antennæ reaching to about the middle of the mesonotum ; joints globose, subequal, the last four being larger and more oblong, especially the apical one, which is prolonged into a short blunt point ; basal joint much larger and more globose than the others ; sense organs, insertion and position of the pile and bristles on the antennæ as in *C. brumalis ;* mesonotum black, shining, pile black ; sides of prothorax with a bunch of black bristles ; pleuræ naked ; mesopleuræ whitish ; abdomen black, shining ; pile on posterior anal segments dirty white, longer than on other parts, central portion of each segment of venter black ; legs black ; tarsi distinctly yellow, infuscated at tips, each joint with a graduated row of bristles on the inner plantar surface ; empodium

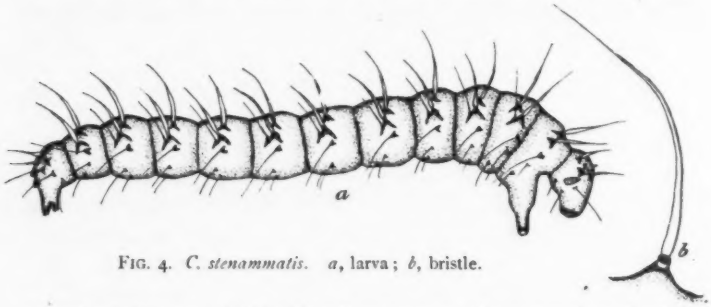


FIG. 4. *C. stenammatis*. *a*, larva ; *b*, bristle.

strap-shaped, curved, bristly-fringed on convex side ; neuration of wings differing from that of *C. brumalis* in the following points : subcostal cell single ; only one anal vein ; no obsolescent veins in cubital or discal cells ; anterior costal cell very narrow ; juncture of the veins at base of wings more distinct.

♂ Coloration practically as in female, pile on entire insect longer, denser and more erect than on female ; the basal joint of antennæ as usual much enlarged, globose ; joints 2–10 inclusive subglobose, graduating in size toward apex and becoming somewhat oblique ; joints 11 and 12 much elongated, subequal, tapering somewhat toward apex ; joint 13 ovate-oblong, smaller and shorter than the apical one. Plumosity, pilosity and sense organs of antennæ as in *C. brumalis ;* also neuration of wings as in female.

Larva : 5–6 mm. long, bristly, and minutely black spinulose ; cylindrical, tapering slightly from the metathoracic segment toward base, tapering rapidly toward the head ; dirty white. Head dark brown, eye spot and mouth parts black ; tips of thoracic feet armed with a circlet

of black claws; anal feet fleshy, armed with two rows of black claws. Chætotaxy of lateral half of head and body as follows: head ornamented behind with one honey yellow, semitransparent, barbellate bristle, in front of which is a large short simple spine, with white bulbous base, in addition to a few simple bristles. Body with three longitudinal rows of barbellate bristles, dorsal bristles honey yellow, enlarged in center; thoracic pair much enlarged and shorter than the others; the dorso-lateral bristles rise from double tubercles, which are simple on the last two segments; anterior limbs of these tubercles tipped with long honey yellow bristles; those of the posterior limbs short and black; lateral row of small, simple tubercles tipped with dark brown bristles; then subventrally are several scattered simple bristles.

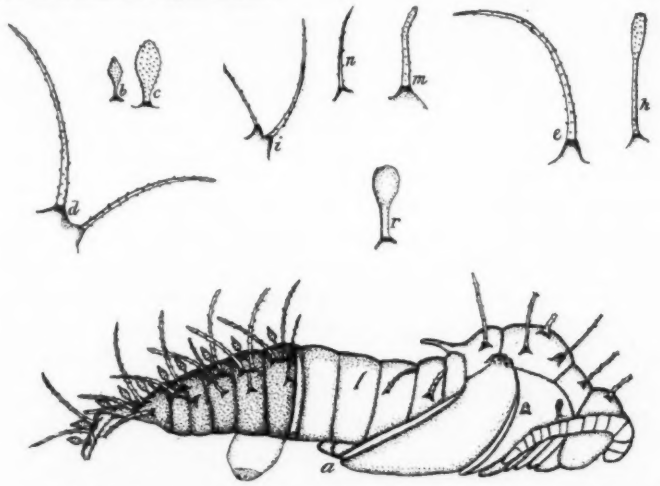


FIG. 5. *C. wheeleri*. *a*, pupa; *b*, dorsal bristle; *c*, enlarged prothoracic dorsal bristle; *d*, furcate tubercle with its two bristles; *e*, lateral bristle.

C. texanus. *h*, dorsal clavate bristle; *i*, lateral furcate tubercle with its bristles; *n*, small lateral bristle; *m*, characteristic spine of pupa; *r*, thoracic stigma of pupa.

Pupa: 2.5–3 mm. long, pale yellow, head darker. Thorax with four large barbellate spines on each side and several small ones with mucronate tips; anterior abdominal segments which are not incased in larval skin, with five longitudinal rows of barbellate spines; the spines of rows 2 and 4 longer and larger than the others. Ventrally is a row of very short spines with mucronate tips. Anal segment forked with two finger-like bodies at base of furcation. These tips were divergent in life and probably serve to retain the larval skin on the anal segments as the spines are obsolete under this skin. These appendages were observed in all the species of which puparia were seen. Duration of pupal stage 8–10 days.

The larvæ are gregarious and live on the under side of dry cow dung from August to December, but are more abundant during November and December, though not so common as *C. brumalis*. The larvæ of both species are sometimes found under the same piece of dung but usually each species is found alone. This species is described here at length as Mr. Coquilett has given only a brief description of the male.[1]

CERATOPOGON STENAMMATIS, sp. nov.

Larva: 5 mm. long, anal segments with two cylindrical feet, tips armed with black claws; feet .15 mm. long; thoracic legs .45 mm. long; color dirty white; head and mouth parts dark brown. Head with two smooth, short, slightly enlarged bristles, one anterior and the other posterior, to the typical head spine, besides a few scattered simple bristles. Either side of body with two longitudinal rows of elongated setigerous tubercles, the dorsal row with only one hyaline acerose bristle to each tubercle, the lateral row with two bristles to each tubercle, one from apex similar to dorsal bristle, the other simple and of a brownish color and from the posterior side of the tubercle; there are besides several scattered simple bristles along the ventral and subventral surface.

The specimens were received from Dr. W. M. Wheeler, who found them in the nest of an ant (*Stenamma fulvum* subsp. *aquia*) at Colebrook, Conn., August, 1900. They were moving about in the refuse heaped up by the ants in certain portions of their nests. The species seems to be a genuine myrmecophile like the European species (*C. Braueri* Wasmann).[2]

CERATOPOGON TEXANUS, sp. nov.

♂, length 2.5 mm.; wing, 1.3 x .4 mm.; ♀ length 2 mm., wing 1.35 x .5 mm.; wings and entire insect densely pilose; one subcostal cell, third or cubital vein terminating distinctly before the middle of the wing; no white spot at apex of costal vein; halteres white; metatarsi slightly longer than succeeding joint; tibia of the female without lanceolate scales.

♀, head dark brown, pile brown; joint 1 of antennæ large globose; joints 2–10 globose, graduating to globose-ovate, subequal; joints 11–14 ovate-oblong, apical one larger. Sense organs and arrangement of pile as in *C. brumalis*. Second joint of palpus in female much enlarged. (See Fig. 6.) Mesonotum drab, with short, appressed, light yellow pile, intermixed with some stouter black hairs; scutellum drab, with several rows of long black hairs; sides of prothorax with a bunch of hairs; pleuræ naked,

[1] Vide, "Some New Diptera," *Proceedings of the National Museum*, Vol. XXIII., 1901, p. 601.

[2] Vide, "Eine myrmecophile Ceratopogen-Larve," *Wiener Entomologische Zeitung*, XII. Jahrg., 8. Heft (10 October, 1893) pp. 277–279.

dirty white, abdominal segments dark brown with dirty white edges producing a banded appearance, which becomes obsolete on the last segments ; pile short, appressed, grayish, becoming longer toward anal segments ; venter dirty white, densely pilose on sides, each segment with long dark pile in front, becoming light yellow behind ; legs dark, pile yellowish and of varying lengths ; tibia of hind legs with a circlet of short spines at apex ; first joint of tarsus slightly shorter than second ; empodium and neuration of wings as in *C. specularis*.

Coloration of male darker throughout than in female ; antennal joints 11–14 inclusive equalling in length the remaining joints ; first joint globose, enlarged ; joints 2–10 depressed globose, graduating in size toward apex and becoming somewhat oblique ; joint 11 much elongated, nearly twice the length of any succeeding joint ; joints 12–14 subequal, apical one slightly larger and more oval-oblong. Pile, plumosity, sense organs, and neuration of wings as in *C. specularis*. Last four segments of abdomen without the whitish edges, blackish, shining, pile denser and longer, without the peculiar pile on venter.

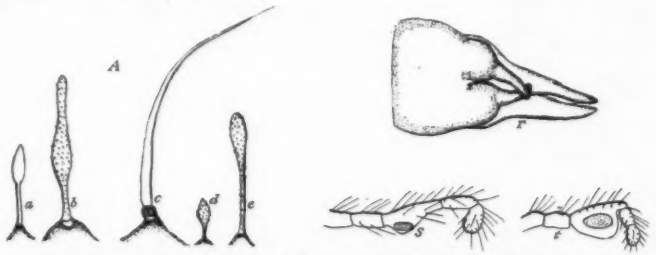


FIG. 6. *A*, characteristic dorsal bristles of each of the five species above described ; *r*, anal segment of the pupa of *C. specularis* ; *s*, palpus of female of *C. brumalis* ; *t*, palpus of female of *C. texanus*.

Larva : Length 5 to 5.5 mm. ; dirty white ; head with two semihya ine bristles ; one in front and the other behind the typical head spine ; chætotaxy for lateral half of body as follows : three longitudinal rows of barbellate bristles ; dorsal bristles hyaline, clavate, minutely tuberculate with segments 5–10 joined at base by a straight black line ; dorso-lateral row of double tubercles, the anterior limbs of which are tipped with curved, semihyaline, broad bristles ; those on posterior limbs short and black ; lateral row of bristles sparsely barbellate, black at base graduating to hyaline at apex ; under the above there are several smaller and subventrally placed simple bristles on each segment ; anal segment with an extra pair of dorsal bristles behind the regular ones. Legs as in *C. brumalis*.

Pupa : 2.5 mm. long, whitish translucent, thorax armed on either side with five yellowish hyaline barbellate spines, the frontal one small with mucronate tip just above and near the base of the antenna. The others on the thoracic dorsum at the corners of a rhomboid ; the anterior spine

truncate with mucronate tip. Stigma black, peduncle semihyaline, the four abdominal segments anterior to the larval skin with three longitudinal rows of spines; those of the dorsal and lateral rows large, hyaline, curved and barbellate, and rising from very prominent tubercles; subvental row of somewhat smaller spines; the tubercles point forward while the spines curve outward and backward. Duration of pupal stage: 7 to 8 days.

The larvæ of this species are gregarious in small numbers beneath the bark of old dead trees in moist places, or on the under side of very damp rotting wood, during December and January. Rare.

CERATOPOGON WHEELERI, sp. nov.

Larva: 5–6 mm. in length; chætotaxy for lateral half of head and body as follows: head with two brown barbellate bristles, one in front, the other behind the typical head spine; besides several scattered simple

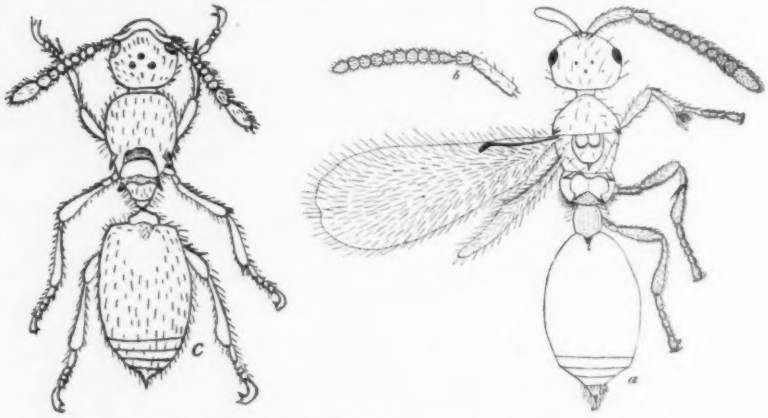


FIG. 7. *a*, ♀ of *Adeliopria longii*; *b*, ♂ antenna; *c*, ♀ of *Solenopsia imitatrix* (redrawn from Wasmann).

bristles; body with three longitudinal rows of barbellate bristles; dorsal row of bristles minutely tuberculate, globose-ovate, hyaline; prothoracic pair much enlarged and more globose; dorso-lateral bristles rise from double tubercles, those on anterior limbs long, light brown and semi-transparent, posterior branches with shorter, jet black bristles; lateral row of small simple tubercles tipped with black shining bristles.

Pupa: 2.5–3 mm. long, head and thorax wine-colored; either side of the thorax with six barbellate spines of varying length and one large tubercle-like spine near stigma, thorax heavily sculptured and minutely spinulose; segments 2 and 3 with a barbellate spine on either side; segment 4 with one small simple spine; other segments unarmed, shining, with small irregular ridges on dorsal portion; anal segment encased in larval skin.

Each of the puparia had been killed by an interesting prototrypid parasite so that no imagines of this new myrmecophilous *Ceratopogon* could be obtained. A number of these parasites were sent to Mr. Ashmead for identification and proved to represent a new genus of the family Diapriidæ. Through the kindness of Mr. Ashmead, his description of this new genus and species is inserted in this article. By way of illustration figures are included of this new species (*Adeliopria longii* Ashmead) and also of the new and closely related species (*Solenopsia imitatrix*) recently described by Wasmann.[1] The Ceratopogon puparia were found December 15, 1900, beneath a stone, in what seemed to be an abandoned ants' nest. The parasites issued, one from the thoracic dorsum of each of the *Ceratopogon* puparia December 31 and lived eight or ten days. This suggests that some of the proctotrupids that have been found in ants' nests and regarded as myrmecophiles may be in reality only parasites of the guests of the ants.

A comparison of the five species above described shows that the larvæ of each have practically the same general size, shape, and color; but the spinous ornamentation is very different, so that they are readily separable by this means alone. In *C. stenammatis* the larvæ differ from the other species in the shape of the anal feet which are cylindrical, short and armed with claws at apex.

The puparia of the Texan Ceratopogons also resemble one another very much. Even the differences in spinous ornamentation are not so marked as in the larvæ. The imagines, however, differ in many essential details, especially in coloration and pilosity. The palpi of both sexes of *C. brumalis* and *C. specularis* are similar, but the female of *C. texanus* has the second joint of the palpus much enlarged and the sense organ on it correspondingly increased in size (see Fig. 6, *s* and *t*); the neuration of the wings of the species is practically identical; the anal extremities of all the puparia which were examined by the writer have the peculiar furcate anal tip and "fingers" described under *C. specularis* (see Fig. 6, *r*). These probably function, as suggested, in holding the old larval skin to the posterior segments of the pupa.

[1] Die psychischen Fähigkeiten der Ameisen, 1899, p. 127 and taf. III., figure 1.

This seems probable also from the fact that the segments encased in the larval skin are smooth, with none of the spines or bristles which appear on the free portions. The tips were observed to be divergent in life but the exact position of the "fingers" could not be determined on account of their being concealed under the larval skin. The dorsal bristles of each of the five species (see Fig. 6, *a*) are the most characteristic and distinguishing features of the larvæ, thus affording a sure and easy means of separating the species. In no case is there any material variation in these bristles for a given species; even when the habitat varies considerably, as in *C. brumalis*, which lives under dung, under rotten wood and in the nests of ants (*Eciton cæcum*), the spinous ornamentation is constant, and the imagines are identical.

A NEW GENUS OF DIAPRIIDS FROM TEXAS.

WILLIAM H. ASHMEAD.

Recently I received from Mr. W. H. Long, Jr., of Austin, Texas, several specimens of a Diapriid, bred by him from puparia of a Dipteron, *Ceratopogon* sp., obtained from an abandoned ant's nest under a stone.

A careful examination of the specimens shows that both sexes are represented and that they will form the type of a new genus allied to the recently established genus *Solenopsia* Wasmann ;[1] but quite distinct in several particulars. At the request of Mr. Long, it is here described to be incorporated by him in a paper that will appear shortly.

ADELIOPRIA, gen. nov. (Diapriidæ).

The two genera may separated as follows :

Females..... 2
Males..... 3
2. Antennæ 11-jointed, the funicle joints 2–7 transverse, the 8th joint quadrate, the club large cone-shaped, unjointed.....*Solenopsia* Wasm.
Antennæ 12-jointed, the flagellum clavate, the funicle joints 6–9 moniliform, the club fusiform, 3-jointed.....*Adeliopria*, n. g.
3. Antennæ 12-jointed ; mesonotum without parapsidal furrows.
Flagellum ending in a 3-jointed club.....*Solenopsia* Wasm.
Flagellum filiform, sparsely, finely pubescent, tapering off at tip, joints 2–11 moniliform, the last joint small, oval.....*Adeliopria*, n. g.

ADELIOPRIA LONGII, sp. nov.

♀ ♂. Length, .8 to 1 mm. Polished, black, impunctate, the metapleuræ clothed with a pale pubescence ; the head and thorax with a few fine long hairs ; the head transverse, the temples obliquely rounded off posteriorly ; scape, mandibles and legs rufo-testaceous ; the legs in ♀ a little the darker with the sutures of trochanters, a dot on knees and the extreme apices of tibiæ yellowish ; in ♂ with the front and middle coxæ yellowish, the scutellum has a large fovea across the base, separated into two by a delicate median carina at base. Metathorax wrinkled with a slight median spine at base, just back of scutellum. Wings hyaline, subemarginate at apex, the apices being cordate ; the venation as in *Phænopria* and ciliate. Abdomen oblong, smooth and polished, the second segment very large, occupying most of the surface ; the petiole longer than thick, furrowed. Type :—Cat. No. 5715, U. S. N. M. (8 ♀ and 2 ♂ specimens) Host. Dipt.; *Ceratopogon wheeleri* Long.

[1] Vide Die psychischen Fähigkeiten der Ameisen, 1899, p. 127.

TWO NEW EMBIIDÆ.[1]

AXEL LEONARD MELANDER.

The singular and primitive family Embidæ of doubtful affinities is represented by not more than a score of living species and a few preserved in amber. Of these nearly one half are known from fewer than five specimens each. Up to 1885, the date of publication of Hagen's Monograph of this family,[2] only fifteen living species had been recorded. Since that time less than a half dozen species have been discovered, so that even now this family remains one of the most poorly represented of the insect world.

The habits of three or four species have been studied to some extent, but only of *Embia solieri* Rambur do we know anything at all approaching the whole postembryonic life-history. It is to Professor Grassi[3] that we owe this contribution. He worked out the internal anatomy of this species and showed how the nests in which it lives are spun by the insect's forefeet instead of by its mouth-parts as had been previously supposed. The object of the present paper is to call attention to two new forms, one from Texas and one from Mexico, and to the peculiar structure of the spinning organ.

Up to the present time only two species have been taken on this continent, one, *Embia* (*Oligotoma*) *hubbardi* Hagen, in Florida and one, *Olyntha salvini* McLachan in Mexico. The new forms probably belong to these same genera, though one of them is here placed in *Embia*. This is done, not because it ought not to be included in *Oligotoma*, where its nearest allies are placed, but because it is believed that the genus *Oligotoma* is untenable. The two genera are separated only by a peculiarity of wing neuration, but Grassi has shown that the adults of

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 16.

[2] Hagen, H., Monograph of the Embidina, *Canadian Entomologist*, Vol. XVII.,, pp. 141–155, 171–178, 190–199, 206–229.

[3] Costituzione e Svillupo della Societa die Termitidi . . . con un' Appendice . . . sulla Famiglia delle Embidini. Catania, 1893.

Embia solieri are wingless, so that in this case the character can not apply. None of the specimens of either of the new species show any traces of wings, although those of the Texan species which were sectioned possess spermatozoa nearly matured. It is interesting to note the distribution of the North American genera. The two *Olynthas* were taken on nearly the same parallel of latitude, and this is also the case with the two *Embias*.

The types of the two new species are deposited in the Museum of Comparative Zoölogy, which also contains the material worked over by Dr. Hagen.

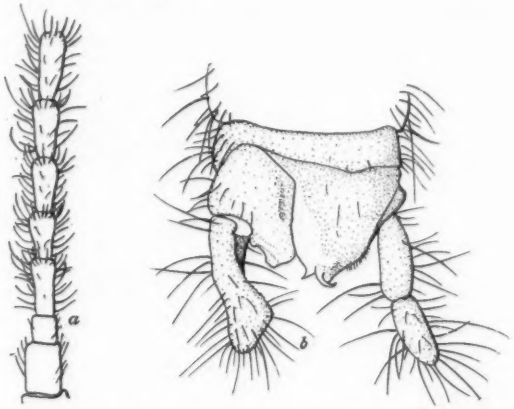


FIG. 1. *Olyntha wheeleri*, sp. nov. *a*, base of antenna; *b*, dorsal view of tip of abdomen.

OLYNTHA WHEELERI, sp. nov. (Fig. 1.)

Length, 6.5 mm. Body black, very lightly subpruinose, punctulate, subglabrous. Insect sparsely covered with fine pale hairs and slender black bristles scattered on antennæ, above eyes, on femora, dense on under surface of four posterior metatarsi, more sparsely on lateral edges of thorax and abdomen, and long on anal appendages. Antennæ somewhat defective, but with twenty joints present, which together are longer than head and thorax; basal joint stout, cylindrical, second one fourth less in diameter, its length and diameter equal, first and second joints piceous, with no erect black bristles, remaining joints dark fuscous with black radiating bristles, third antennal joint equal to two basals, its sides concave, remaining joints elongate pyriform, thicker beyond middle, fourth shorter, fifth to sixteenth subequal, seventeenth to twentieth a little shorter. Labial palpi rather

thick. Maxillary palpi plainly five-jointed, second and third joints subequal, each a little shorter than first, fourth longer than first but more slender, fifth not longer than third plus the fourth, with numerous pale hairs. The bases of the maxillæ and the galeæ are testaceous, remainder of head black. Thorax flattened above, the mesothorax larger than the others, evenly black, no color markings. Legs black, last tarsal joint alutaceous at tip, ungues pale with piceous apex; fore metatarsi greatly enlarged, with pale recurved hairs along edge of lower surface, middle joint with similar hairs: tarsi of four posterior legs slender, metatarsi with a dense brush of thick, short, black bristles beneath, middle joint with paler papilla, third joint slender, long; middle legs slender; posterior femora much incrassated. Left anal appendage one-jointed, but articulated with the body, stout, large, clavate, obliquely truncate at tip, lightly rugose. Right appendage two-jointed as usual, the apical joint shorter and thinner than the basal. Both appendages have many long brown to black hairs. Between the appendages are two triangular lamellæ. The left one testaceous, the right one black and testaceous apically: each armed with an apical spine pointing transversely outward. The median portion of the last ventral segment is placed largely towards the right. From the upper surface the secondary sexual characters present a different aspect. The terminal abdominal segment (10th) is obliquely cleft, the cleavage starting near the left side and terminating nearly midway between the appendages. The left portion is prolonged into a hastate slender projection. Near the base of the left appendage arises a flat lighter-colored triangular process (seen from below as the left triangular lamella). This overlies the hastate projection. The right portion of the last segment terminates in a flattened bifurcate process, the outer tooth of which is bent downward and the upper tooth to the right. The ninth abdominal dorsal segment is very narrow, of about one third the depth of the others: its posterior margin is arcuate, bulging outward on the right side and emarginate on the left.

From above the head is one half longer than broad, wide at the rather prominent eyes and then sloping suddenly forward and less so behind the eyes; hind angles very oblique; head not at all quadrangular in outline; no impressed markings; surface evenly, finely rugulose.

Among other characters, the shape of the head, the structure of the antennæ, the color of the hairs and body and the remarkable sexual organs are peculiar to this species and readily distinguish it from the other species of *Olyntha*.

One wingless specimen from Cuernavaca, Mexico, taken December 26, 1900, by Dr. Wm. W. Wheeler while excavating a nest of *Leptogenys wheeleri* Forel.

EMBIA TEXANA, sp. nov. (Figs. 2 and 3.)

Immature Male.—Length, 5–7 mm. Rufous. Antennæ pale fuscous, when folded back reaching to second pair of legs, filiform with small testaceous setæ. First three joints more or less quadrate, others rounded, first joint rather thicker than the others, a little longer than broad: second the palest, as long as broad: third pale at base, nearly twice as long as broad: fourth and fifth but little longer than broad: remaining joints gradually increasing in length, last three joints twice as long as wide, their diameter nearly equal to that at base. No more than sixteen joints were

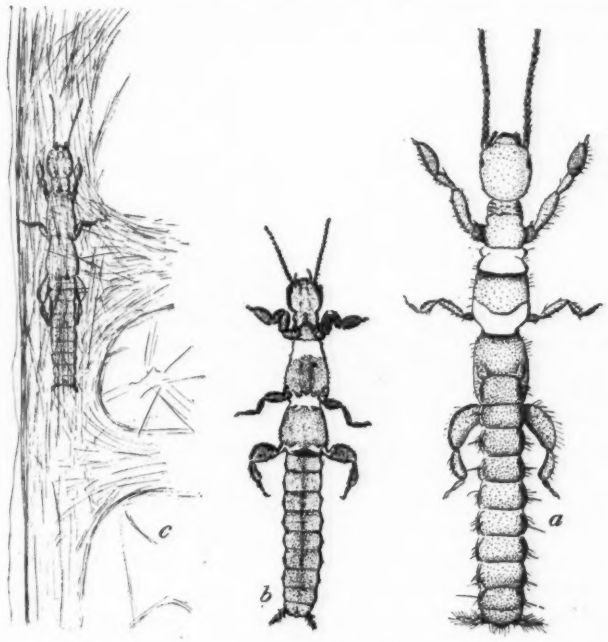


FIG. 2. *Embia texana*, sp. nov. *a*, dorsal view; *b*, ventral view; *c*, insect in web.

counted in any one specimen. Each antennal joint has a small pale space at its base. The 5-jointed maxillary palpi become paler apically and have the first joint connate with the head, second joint very short, third, fourth and fifth gradually increasing in length and decreasing in breadth, fifth a little longer than the two preceding together. Third joint of the labial palpi broad, yellow. Galeæ yellow, prominent, broad. Mentum and submentum rufous, as is a large W-shaped mark on the lower side of the head. Eyes oblique, black, consisting of about fifty facets. Neck tri-

angular beneath, with two small rufous tubercles, between which are the apices of two rufous triangles. The thoracic sclerites transverse, rufous, separated by colorless thin chitin. The sterna are well-marked. Abdominal sclerites more firmly chitinized than thorax and therefore darker red. Anal plate divided, a little asymmetrically, the right piece with a slight subapical notch on inside where there is a transverse carina. If any differences exist in the anal styles the basal joint of the left is a little stouter. Legs rufous except middle and hind coxæ and trochanters, knees, and last tarsal joint. The fore and hind femora are strongly incrassate as are the fore tibiæ and tarsi. Under side of the front metatarsi nearly plane, beset with minute and with moderately long bristles, the longer set recurved at tip. Remainder of legs and body covered with scattered pale yellow

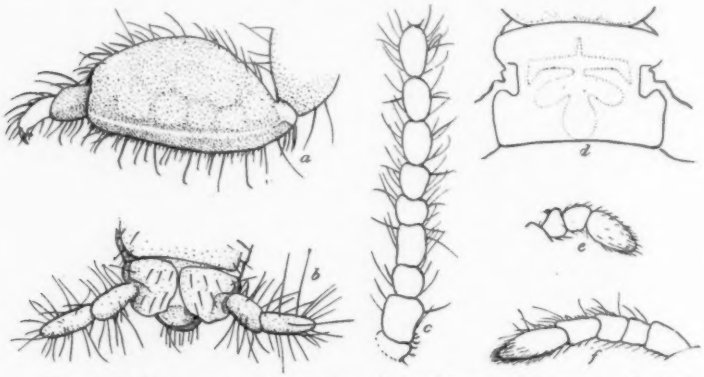


FIG. 3. *Embia texana*. *a*, front tarsus; *b*, tip of dorsum of abdomen; *c*, base of antenna; *d*, prothoracic notum; *e*, labial palpus; *f*, maxillary palpus.

bristles which are longer on the anal styles. Upper side of head with three clear "ocellar" spots, a Y-shaped mark confluent with an inverted heart-shaped, basal space, on each side of which is a granulate clavate mark. The stem of the Y continues along the middorsal line of the thorax and more faintly along the basal segments of the abdomen. Upper side of the thorax with an inverted "fleur-de-lis"- shaped, granulate, clearer mark. These color-marks are sometimes faint. No traces of wings.

Eight specimens taken during the winter months, from November to March. Austin, Texas.

The following are the chief characters which separate the related species from the above-described form:

Embia (*Oligotoma*) *cubana* Hagen. *Winged male.* Head cut straight near the prothorax; last palpal joint long-conical; between anal styles above is a short bent process, on the left side

of which is a conical lobe, short, open at tip. *Wingless female.* Antennæ 18-jointed; last ventral segment not divided; above between appendages is a small thin elongated lobe.

Embia (*Oligotoma*) *hubbardi* Hag. *Winged male.* 4 mm. Head cut straight before prothorax; second joint of antennæ very small, annular; third joint as long as two basals, thicker on tip; fourth and fifth similar to third but a little shorter; abdomen pale brown.

Embia (*Oligotoma*) *insularis* McLachl. *Winged male.* Sixth antennal joint as long as third; apical joint of labial palpi ovoid; abdomen pale dirty brown; last ventral ending in a bottle-shaped tube which is turned to the left and partly surrounded by a horny hook.

Embia texana is very susceptible to differing degrees of humidity and therefore cannot be found at all seasons of the year. After the ground has been moistened by the winter rains the Embiids were found, the last one being taken during March. As soon as the overhead sun dries up the ground these insects are never seen, having burrowed deeply into the soil. One specimen was taken while ripping the bark from a fallen tree, all the others were found under stones; and of these one was found in company with *Formica fusca* L. var. *gnava* Buckley, though its association with the ants was accidental, no doubt, as was shown by its hurry to escape. In this connection attention may be called to the occurrence of the *Olyntha* in the nest of *Leptogenys*. *Embia texana* is apparently not rare in this vicinity, two having been taken in a single day, though from its neat manner of concealment and its limited time of appearance it is found only by the merest chance. The species has been taken in localities about the city of Austin separated by over ten miles, places of different character, one a sandy tract, and the other a limestone hill.

The insects live singly in silken webs, spun by themselves. These webs are tunnels in some niche of the rock which shelters them, or spun between the grains of soil. They are an inch or more in length, and closed at one end, their diameter a little greater than the insect's body. Sometimes a small flat web is spun beneath the stone. The tunnels are provided with side-

passages ending, in the case under observation, blindly (Fig. 2, *c*). When the Embiid wishes to turn in its passage it backs partway into a side tunnel until its head is free to point the other way. This is not universal, however. *Embia* was several times seen to turn on itself, as its very supple body and unchitinized joints permit it to place the thorax in a line parallel with and touching its abdomen. During the daytime *Embia* seemed loath to quit its tunnel, and when forced out would always return to it in a few minutes. When in the tunnel it is completely concealed. This accounts for the few specimens, as all were taken when driven from their retreat on the removal of their stone.

It was stated by Hagen that the web is used in ensnaring prey. Its texture is far too delicate for that purpose, even were its size larger. *Embia's* silk is very different from spiders' webs. It is much more frail and of an opalescent whiteness which renders it distinguishable, after practice, from the spider webs common in the same situations. Grassi states: "Evidently the gallery serves to protect the body from too excessive transpiration, and to keep about the *Embia* an atmosphere not too dry," but it is difficult to see how a net can prevent excessive transpiration in a dry climate like that of Austin. The tunnels probably serve merely as a retreat. The insect seems entirely contented when at home and touching the meshes of its web, so that it may be described as strongly, positively thigmotactic. Indeed, it was frequently observed to stretch its front feet outward, in order to press its back against the soft silk.

When the web was touched, the Embiid darted out, sometimes head first and sometimes crawfishing. Its backward movements, however, are very different from its normal walk. In moving ahead *Embia* walks with a sinuous motion, bending its supple body slightly to accommodate the motions of its legs, and covering about two thirds of an inch, or less, in one second. Its recoils are more of a scurry from danger. It then travels a full inch in a second and in a straight path, though it never goes beyond an inch in any single dart. While walking, the abdomen is carried in a very unstaphylinid-like manner, the central part being elevated and the tip depressed. *Embia* was never observed to jump, though the incrassate and muscular femora would at first sight seem to indicate that habit.

One of the *Embias* was kept in a small culture dish with a few grains of sand. The first night it spun along the edge of the glass a web in which it remained the two following days. Then it discovered the sand in the center of its room and spun another gallery between the surface of the glass and the sand. In this position it was absolutely concealed, having completely covered the web with the small amount of sand present. *Embia* is exclusively nocturnal, at least in the nymphal state. Although the tunnel was in great part completed in a single night additions were made to it on several succeeding nights.

At first sight the silk resembles a very thin, pale gossamer, but when viewed under a strong magnification the individual threads are seen to be arranged in fine bundles of varying thickness. Most of the threads in a bundle lie parallel with one another, but the bundles contain a few curled threads also. The individual threads vary in diameter.

In making its web *Embia* does not use its mouth—notwithstanding Hagen's statement that spinning organs are present among the mouth parts—but it uses instead, its forefeet, as Grassi had shown. No other animal has developed spinning organs in this position. The silk is produced by large glands occupying nearly the whole of the enlarged metatarsus. The underside of this joint is provided with a dense mat of small short bristles, and interspersed among them are the longer spinning bristles, each of which is hollow and slightly recurved at the tip and contains a tenuous duct from the gland. While spinning, *Embia* very rapidly moves its forefeet, either singly or both together, now reaching out directly in front, now to the side, with a movement which reminds one of a cat toying with a string. Each time it touches a surface it attaches a bundle of silk. As the bundles are of varying sizes it would seem that sometimes all the glands function, at others only one.

While spinning its cylindrical retreat, *Embia* slowly rotates on its longitudinal axis. When within its nest it does not find it inconvenient to rest with head downward. It was seen resting on its side or back more frequentiy than with its ventral surface down. The soothing touch of its web seems to be suffi-

cient to insure a feeling of security no matter how its body may be placed. In its natural habitat the insect is normally found clinging to the under surface of its sheltering stone. But when driven from its gallery, *Embia* rebels if placed in an inverted position and rights itself immediately by wriggling the tail and clutching with the forefoot, which probably at the same moment emits a strand of silk.

The antennæ of *Embia* appear to be less sensitive to touch than the body. The insect would frequently butt against some obstruction notwithstanding the warning received through the antennæ. On one occasion the warning was insufficient to prevent *Embia* from walking into a drop of honey, and after an enforced bath the insect seemed more solicitous of its forefeet than of the rest of its body, carefully eating off the honey from their plantar surfaces. When about to clean its antennæ *Embia* rapidly turned its head and thus brought one of these organs under the body and then standing over it gradually drew it forward, at the same time eating off the honey. This was the only time the insect was observed to eat anything of a vegetable origin, and then it was only with great reluctance. On several occasions small pieces of a worm and a fly attracted its attention but after a few nibbles it would run away. The abandoned web at the edge of the jar seemed to be a more toothsome morsel, for *Embia* was frequently observed nibbling at it.

The spinning glands of *Embia* (Fig. 4) are unique and without parallel. The silk is produced in the thickened anterior metatarsi within chambers and conducted outside to the recurved hairs at the edge of the plantar surface. The chambers of the metatarsus which may be seen shining through the chitinous integument, appear more or less regularly arranged in three longitudinal layers, one next to the sole, a second in the middle, and a third dorsally. In each series there are, roughly estimated, about twenty-five chambers, placed about three abreast. Thus in the whole joint the chambers number between seventy-five and eighty. The cavities are nearly all of the same size—about sixty micra in diameter—though the outer chambers are somewhat smaller. Each chamber is more or less cuboidal and bounded by a single layer of epithelium. This is for the most part quite

thin and flattened in the central part of the faces, but becomes columnar at the corners. Cell-boundaries are not easily seen. The large central space of each chamber is filled with a colloid secretion, more or less shrunken in alcoholic material. This substance, the silk, is carried to the tips of the hairs through ducts of varying length, one from each chamber (Fig. 4, *b*). The terminal hairs (Fig. 4, *a*) are arranged in a row around the edge of the plantar surface of both the metatarsus and the second tarsal joint. Though the latter is devoid of any secreting gland it possesses several ducts leading through it from the metatarsus. Some of the ducts lead along the periphery of the joint, others

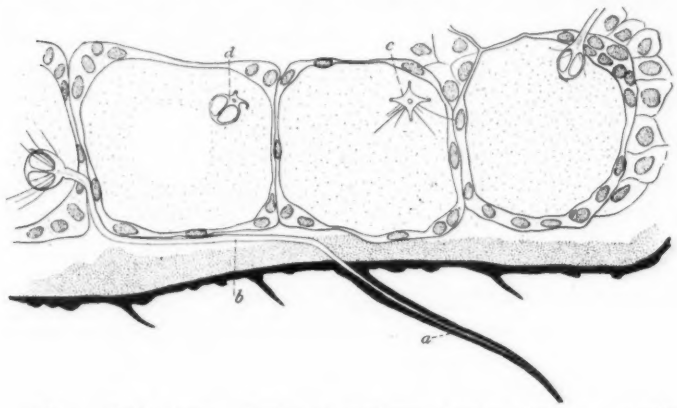


FIG. 4. *Embia texana*. Portion of metatarsus, showing glands; *a*, spinning bristle; *b*, duct of silk-tube; *c*, ampulla at base of duct.

pass between the gland-chambers. The foot-tendon lies in large part between the two lower series of glands.

In the glands, each duct arises from a remarkable ampulla (Fig. 4, *c*, *d*, *e*). The wall of the duct suddenly becomes thickened, and then subdivides into four or five rays, which are continued in the form of the equidistant meridians of a sphere and meet again in an end-plate opposite the point of subdivision. Thus the lumen of each duct terminates proximally in four, or rarely five, large, elliptical or ovoid, lateral openings. At the base of each opening is a radial arrangement of fine processes, possibly the expression of the silk being drawn into the lumen of the tube.

UNIVERSITY OF TEXAS, AUSTIN, TEXAS, March 22, 1901.

Postscript.

Since the foregoing account was written we have met with *Embia texana* a number of times, while on two occasions several specimens were associated in the same nest. In the former of these cases the retreat was the combined efforts of six individuals, in the latter but three specimens resided together. These family homes are larger than those of the solitary *Embias*, extending for several inches and consisting of a large mass of silk. As none of the specimens observed show any trace of wing-formation, our previous conclusion, that this species is wingless, is probably correct.

Austin, Texas, March 22, 1902.

THE NESTING HABITS OF ANTHIDIUM.[1]

AXEL LEONARD MELANDER.

The care and forethought which an insect shows in providing for an offspring which in most cases she will never see have always excited the interest of biologists. Among these insects the solitary bees have especially attracted the attention of observers on account of their varied habits of nest construction. Here, in a group of insects more or less homogeneous, we find the style of architecture under such differing forms as the fence-rail excavations of the carpenter-bees, the thimble-like nest of the leaf-cutters, or the *Andrena*-burrows in wayside paths.

The genus *Anthidium* affords no less interesting methods of house construction. For many years the habits of a few species of this genus have been well known, but they constitute but a small contribution when the whole number of species is considered. In a recent monograph of the European species of *Anthidium* Dr. Henry Friese estimates the total number of species of the world at between five and six hundred. Already some two hundred and twenty have been described, but of these the habits of the two hundred are yet to be made known.

Anthidium is a genus of fixed morphology, that is the species rather markedly resemble one another. All have the abdomen destitute of hair above, and brightly ornate with yellowish markings. Notwithstanding this similarity in appearance the habits of the species are quite different. Two distinct methods of nest-building are presented, from one or the other of which the species, at least so far as known, scarcely ever depart. The French entomologist, Fabre, has tersely called these two categories, those of the "Cottonniers" and of the "Résiniers."

The cotton-workers do not show much deviation from the habits of other bees and might be considered to possess the primitive nesting-habit of this genus. Among bees the female either digs a hole in the ground or, more usually, occupies one already dug. Apparently all that is sought is a convenient

[1] Contributions from the Zoölogical Laboratory of the Uninversity of Texas, No. 12.

nidus; the form is of small moment. *Anthidium* has been known to choose abandoned burrows of other bees, or the excavations made by Scarabæid larvæ. There are several cases recorded where even key-holes have been utilized. In other species the nests are placed, not in the ground, but in hollow plant stems. In all these cases the nest is lined with a hard compacted cottony down scraped from the stems of pubescent plants by means of the dentate mandibles of the female.

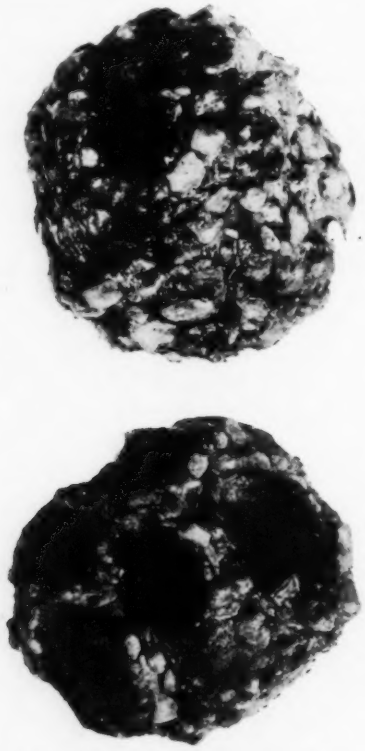


FIG. 1. Nest of *Anthidium texanum* Cress.

With the "Résiniers" the habits are to some extent different. They have the peculiar habit of constructing their nests with the aid of the resin of various conifers. In addition to this some

of the species invariably seek out the empty shells of different Helices wherein to start their future family.

Thus far, the habits of but three of the forty-one North American species of *Anthidium* have been made known. Of these *emarginatum* and *paroselæ* are "Cottonniers," and construct their nests normally in dry sand-banks. The third species, *consimile*, builds among the branches of shrubs or in crevices in rocks and has habits somewhat resembling those of the resin-worker, *texanum*, here to be described.[1]

That *Anthidium* presents the two types of nest construction in both the Old and the New World shows these to be very ancient habits whose divergence occurred at an indefinitely remote period.

While collecting insects in January, on the south side of the Colorado River, a few miles west of Austin (Texas), at an altitude of about eight hundred feet, a nest of what proved to be *Anthidium texanum* was found.

This structure was fastened to a branch of cedar (*Juniperus virginianus* Linn.) about eight feet from the ground. In appearance it was like a small rounded conglomerate with a greatest diameter of twenty-three, and a least of eighteen millimeters. This curious nest consisted of small pebbles of limestone, from one to three millimeters in thickness, securely cemented together with an amber-colored resin, presumably derived from the cedars. Its weight after the bees had transformed was five grammes.

In this mass were six pupal cells. They consisted of a tough, chestnut-brown membrane, more or less transparent so that the pupæ within could be seen. They were four and one half millimeters in diameter, about ten millimeters deep, with a rounded bottom, flat top, and slightly narrowed in width above. The flat top consisted of tougher material, in large part spun in concentric rings around a whitish, conspicuous mammilla. This projection, which is characteristic of *Anthidium*, contained a central hole, and canal to the interior.

[1] In the description of *consimile*, Dr. Davidson did not state of what material the cement was composed. Since then he has informed me that the species builds at times miles from any pines, so that then, at least, other plants probably furnish the resin. Another species, which unfortunately was not identified, was found building in pine resins on San Jacinto Mt.

The arrangement of the pupæ deserves notice. Two were entirely enclosed in the resinous mass except at their mammillated end. The three adjacent were more exposed, all on the same side from the two enclosed cells, and arranged in a row parallel with that of the two, while the sixth, which contained the only male, was quite uncovered and placed on another side of the nest. This also was the last to transform. The five contiguous cells contained females. All the pupæ were oriented alike; also, the head of the pupa was nearest the mammillated end. The first bees, two in number, emerged on May 16th, and immediately crawled back into their cases head first. The next transformed on the 19th, the fourth and fifth on the 25th, while the male did not appear until June 4th. Each imago repeated the habit of the former ones, by crawling head-first into its vacated pupa-case. The adult bees seemed to have eaten the flat end of their envelope in getting out, for no traces of it could

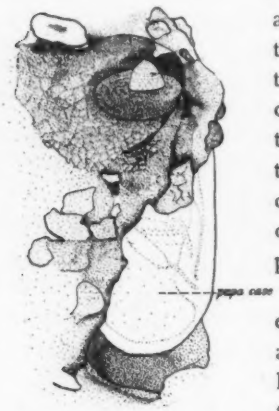


FIG. 2. A single pupa cell showing the mammilla.

FIG. 3. *Anthidium texanum* Cress.

be found afterwards in the breeding-cage. No cottony pubescence whatever was employed in the nest-construction. The

Californian *consimile* uses granite pebbles as the basis for its nest whereas *texanum* employs limestone, there being no granite at Austin.

The adult bees are quite rare, apparently only two males having been taken previously, by Mr. G. W. Belfrage, in Bosque Co., Texas. These are the types in the collection of the American Entomological Society in Philadelphia, and have been compared with the present specimens to insure the accuracy of the specific determination. The females differ from Cresson's description of the male in having the clypeus largely black; the only yellow occurs in the extreme lateral corners. The sixth abdominal tergite is wholly black. The mandibles also are black and their apical margin is evenly truncate, whereas in the male they are apically emarginate on each side of a median tooth.

Below is appended a list of the species of *Anthidium*, the habits of which are known, and also the papers referring to these. The habits of a number of species are reviewed by Dr. H. Friese in his Apidæ Europæ, Vol. IV. Many interesting notes on the flowers visited by *Anthidium* are given in Knuth's Blütenbiologie, especially in Vol. II., pt. 2, p. 601.

"COTONNIERS."

cingulatum: Fabre, Souveniers Entomologiques, Vol. IV.

diadema: Fabre, Souveniers Entomologiques, Vol. IV.; Rudow, Soc. Entom., 1887–1888.

emarginatum: Davidson, Ent. News, 1895.

florentinum: Rudow, Soc. Entom., 1887–1888; Fabre, Souv. Entomol., Vol. IV.; Rudow, published by the author, Perleberg.

lituratum: Rudow, Soc. Entom., 1887–1888.

manicatum: Rudow, Soc. Entom., 1887–1888; Rudow, published by the author, Perleberg; Verhoeff, Zool. Jahrb., Syst. VI.; Fabre, Souv. Entom., IV.

montanum: Dalla Torre, Entomolog. Nachricht., 1880.

oblongatum: Rudow, Soc. Entom., III.; Xambeu, Bull. Soc. Ent. France, 1896.

paroselæ: Minnie Newberry, Psyche, 1900.

scapulare: Fabre, Souv. Entom., IV.

"RÉSINIERS."

bellicosum: Fabre, Souv. Entom., IV.

consimile: Davidson, Ent. News, 1896.

contractum: Lichtenstein-Montpellier.

latreilli: Fabre, Souv. Entom., IV.

quadrilobum: Fabre, Souv. Entom., IV.

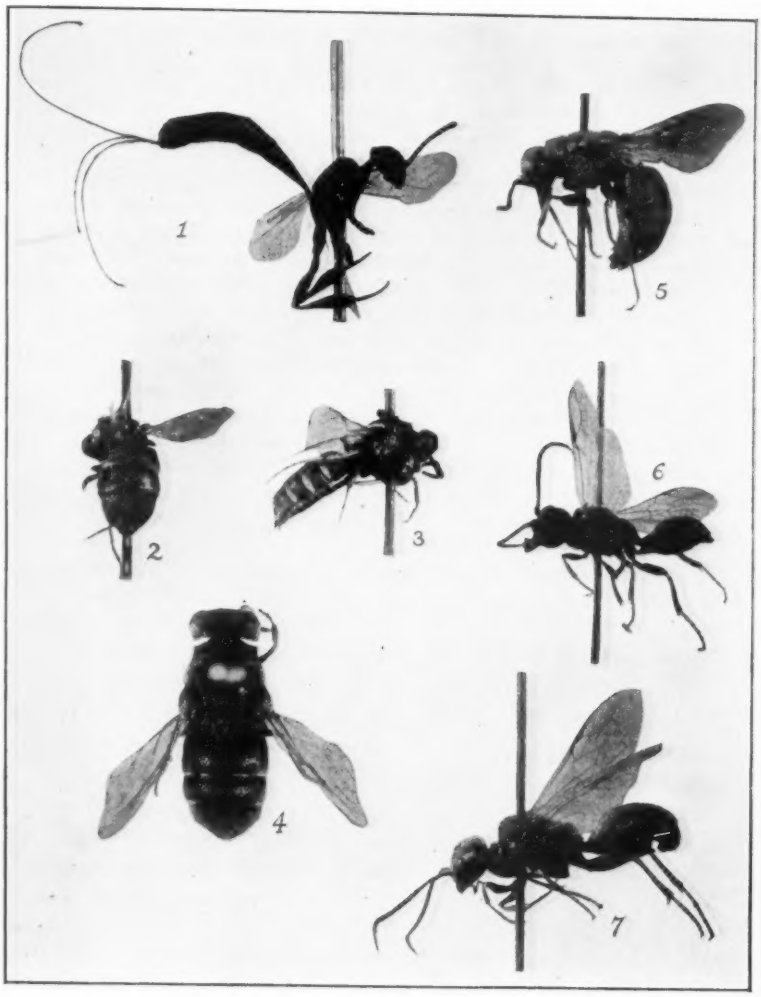
septem-dentatum: Fabre, Souv. Entom., IV.; Xambeu, Bull. Soc. Ent. Fr., 1896.

sticticum: Lucas, Explorat. d'Algérie, 1846; Tosi, Bull. Soc. Ent. Ital., XXIX.

strigatum: Friese, Apidæ Europæ, Vol. IV.; Kirschbaum Nassanisch. Jahr., 1871.

texanum: Described above.

THE UNIVERSITY OF TEXAS,
AUSTIN, TEX., Nov. 14, 1900.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EXPLANATION OF PLATE.

FIG. 1. *Gasteruption pattersonæ*, ♀.
FIG. 2. *Parnopes westcottii*, ♀.
FIG. 3. *Parnopes westcottii*, ♂.
FIG. 4. *Parnopes aglaspidula*, ♀.
FIG. 5. *Parnopes aglaspidula*, ♀.
FIG. 6. *Trigonalys hollensis*, ♀.
FIG. 7. *Trigonalys hollensis*, ♂.

All figures are enlarged; reproduced from photographs made by the authors.

NEW SPECIES OF GASTERUPTION, TRIGONALYS, PARNOPES AND PSAMMOPHILA.[1]

AXEL LEONARD MELANDER AND CHARLES THOMAS BRUES.

GASTERUPTION.

GASTERUPTION (FŒNUS) PATTERSONÆ, sp. nov.

Female.—Length, 11 mm., ovipositor 10 mm. Body ferruginous. Head black. Dorsum of thorax; dorsal line on first abdominal segment, extending nearly to tip of segment; dorsal spots on second and third segments, somewhat indistinct on third; then a dorsal line which widens out, so that it covers the last segment almost completely; piceous. Ovipositor shields very dark fuscous. Legs ferruginous, hind tibiæ slightly darker outwardly and toward apex, and banded with white near base. Clypeus and mandibles ferruginous, the latter black at extreme tip. Palpi rufous, last joint black. Antennæ about as long as head and thorax, fuscous; scape rufous; flagellum black at base and lighter below except on apical joint. Wings hyaline, nervures and stigma fuscous. Head, thorax, and posterior coxæ silvery pubescent. Clypeus quadridentate, fringed with golden hairs. Front punctate above antennæ, and very finely and somewhat obsoletely, transversely aciculate. Prothorax rather deeply and finely punctured. Pleuræ rugose; dorsum and metathorax transversely rugose aciculate. Abdomen impunctured, smooth, not very shining; strongly compressed at base, widest at third segment which is one-half as wide as high. Ovipositor longer than abdomen, alutaceous.

Described from a female specimen, collected by Miss Rose Patterson, August 11, 1898, at San José, California.

This species may be readily recognized by the entirely ferruginous pleuræ, legs, and antennal scape and by the very slight amount of black upon the abdomen. It is the only species recorded from California.

Mr. Ashmead[2] has pointed out the synonymy of *Fœnus* Fabr., and *Gasteruption*, which was described two years previously, viz., in 1796, by Latreille.

TRIGONALYS.

The species of *Trigonalys* from the United States and Canada so far known may be recognized by the following artificial key:

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 11.

[2] Classification of the Ichneumon Flies; *Proceedings United States National Museum*, No. 1206, July, 1900.

Insect almost wholly black..... 2
With conspicuous yellow markings..... 3
2. Abdominal spots larger ; body less coarsely punctured ; subtegular tubercle black;
♂, 9.5 mm .... *pullatus* Schuck.
Abdominal spots smaller; body strongly punctured ; subtegular tubercle yellow ;
♂, 11 mm..... *hollensis*, var. nov.
3. Second abdominal segment fulvous with a large, subquadrate, black spot on each side, and a transverse lateral white spot at tip..... *pulchellus* Cress.
Second abdominal segment black with at least a tranverse yellow stripe..... 4
4. Scutellum wholly yellow ; 7 mm..... *sulcatus* Davis
Scutellum somewhat black in middle ; larger..... 5
5. Coxæ and femora black ; wings with subapical cloud..... 6
Coxæ yellowish, femora dark fuscous ; wings fuscous along costa . *costalis* Cress.
6. Head confluently punctured.....*nevadensis* Cress.
Face above antennæ, and vertex impunctate..... *canadensis* Harntgn.

TRIGONALYS PULLATUS Shuck. var. HOLLENSIS, var. nov.

Male.—Length, 11 mm. Black, coarsely punctured, with almost sericeous, short white pile proceeding from each puncture, and covering whole insect. Antennæ with twenty-four joints, black, filiform, slender, the middle joints stouter than the others, the joints evenly decreasing in length to the pointed tip ; scape black. Head strongly, closely punctured ; antennæ inserted in upper portion of transverse broad depression. Mandibles prominent, broad, with three or four well-marked teeth. Maxillary palpi slender, six-jointed ; labial palpi consisting of three stouter joints, the last securiform. Tegulæ piceous ; tubercle below tegulæ yellow. The punctation of the thorax is more rugose than that of head or abdomen. Parapsidal depressions well marked, as is also a central longitudinal groove. Scutellum prominent, with median longitudinal groove, and deeply foveate at sides. Postscutellum strongly fossate at sides. Metathorax rugosely reticulate on posterior declivity. First segment of abdomen short, triangular, second largest, four times length of first, third one half length of second, rest of abdomen incurved, not visible from above, fourth and fifth segments subequal, sixth provided with a rough series of small tubercles. At the apex of the abdomen are two short claspers. Second, third and fourth segments each marked with an inconspicuous yellow lateral spot near posterior margin. First and second ventrals equivalent to first and second dorsals, remaining segments much smaller on account of the infolding of the abdomen : second ventral provided with a subapical, flat, median tooth, beyond which the abdomen is deeply notched ; last ventrals carinate in middle. Legs marked with yellow as follows : exterior faces of all tibiæ, broadly interrupted on middle pair and narrowly on posterior ones, exterior face of front metatarsi, and exterior two thirds of middle and hind metatarsi. Pectus swollen, deeply, longitudinally grooved. Wings hyaline at base, with a slightly infuscated cloud on apical third : subcostal and externo-

medial nervures strong, piceous, remaining veins weaker, interrupted in places; second and third submarginals subequal, second narrowed towards marginal cell, third subquadrate.

Female.—Differs from male as follows: Length, 8½ mm. Antennæ twenty-three-jointed. Second abdominal segment only with a small yellow posterio lateral spot. Abdomen without the secondary sexual characters of the male, shaped much as in *Nomada*, with four genital filaments, the outer pair of which are fimbriate. Four posterior tibiæ with only a basal yellow band, the hind ones with only slight indications of the striping. The second submarginal is longer than the third and subquadrate.

The two specimens were taken at Woods Holl, Mass., during the latter part of July. They had the peculiar habit, noticed in *canadensis* by Dr. Geo. W. Taylor, of alighting on the leaves of low trees.

The stripes of the four anterior tibiæ show a twisting from the knee towards the front. In the male there is also a small basal stripe almost contiguous to the anterior side of the stripe on the hind tibiæ.

Inasmuch as Mr. Shuckard's three-line description of the color markings of *pullatus* can be applied with quite as much certainty to *Oxybelus quadrinotatus* or even to *Eristalis tenax*, it seems advisable at least to redescribe the present form under the name given. Mr. Wm. H. Ashmead writes that he has a specimen of this rare wasp from Minnesota, which in consideration of the type-locality, North Carolina, shows a wide distribution for this species. The differences given in the table between *pullatus* and *hollensis* are not positively stated. The following are the North American species of *Trigonalys* from Mexico northwards.

pullatus Schuckard,	1841,	N. Car., N. J.,	9.5 mm.	♂
costalis Cresson,	1867,	Mass.,	9. "	♂
pulchellus Cresson,	1867,	W. Virginia,	8.8 "	♂
nevadensis Cresson,	1879,	Nevada,	8.8 "	♂, ♀
canadensis Harrington,	1896,	Victoria B. C.	10.5 "	♀
canadensis Harrington,	1898,	Gabriola Is., B.C.	11.4 "	♂
sulcatus Davis,	1897,	New Jersey,	7. "	♂
hollensis n. var.,	1900,	Mass.,	11. "	♂
			8.5 "	♀

PARNOPES.

In 1874 Mr. F. Smith published the description of *Parnopes chrysoprasina* from North Carolina. Since that time no one has found this insect, though the fact that the species of this genus

are widely distributed is not to be doubted. But four North American species have hitherto been known, and these, with the two new species, may be readily identified by use of the following dichotomy:

Postscutellum excised 2
Postscutellum entire ..... *edwardsii* Cress.
2. Flagellum black; legs metallic; tegulæ green..... *aglaspidula*, sp. nov.
Flagellum and legs reddish; tegulæ in part testaceous 3
3. Face and metapleuræ bare..... 4
Face and metapleuræ with silvery pubescence; wings hyaline..... 5.
4. More or less bronzed; tibiæ brown..... *festivus* Ckll.
Green; tibiæ with greenish tint ..... *chrysopasina* Smith.
5. Postscutellum black, wings hyaline; abdomen green or bronzed; 6.5 mm. *westcottii* sp. nov.
Postscutellum fulvous; apex of wings somewhat infuscated; abdomen bluish; 9 mm,..... *fulvicornis* Cam.

PARNOPES AGLASPIDULA, sp. nov.

Female.—Length, 10 mm. Bright metallic blue-green. Rugosely-closely punctured. Nowhere densely pubescent, but with a few scattered white hairs, more closely placed on sides of posterior margins of abdominal segments. Antennæ piceous, the scape with greenish reflections. Face above antennæ smooth, excavated, with impression of an equilateral triangle the base of which lies between the bases of the antennæ and from the apex of which arises a vertical line passing nearly one half way to the anterior ocellus. In *P. edwardsii* there is no such sculpturing. Occipital punctures confluent. Mandibles rather robust; black, except for piceous central space. Clypeus concolorous with face, its black apical margin truncate and devoid of hair. Proboscis reaching to first abdominal segment. Thorax with larger punctures than head, the punctures becoming stronger posteriorly. The median prothoracic impression is almost obliterated, as are also the parapsidal grooves. Between the parapsidal grooves the thorax is almost black, likewise the scutellum and postscutellum. Postscutellum with a narrow incision extending about one third the distance into the disc, its sides straight in one specimen and bowed outwardly in the other. Tegulæ narrow, wholly blue-green, with less strongly-marked punctures than thorax. Metathoracic angles prominent, with reticulate markings. Abdominal segments violet-blue on anterior and posterior margins, green in middle portion; with no apically depressed bands: apical segment with a median carina terminating anteriorly in a narrow, smooth space and posteriorly separating the two subapical oblique grooves, the margin denticulate, with the stronger teeth, about fourteen in number, in a middle series. Venter piceous, smooth. Legs concolorous with body, though gradually lighter in color towards end: knees, apices of tibiæ, and tarsi lighter. Wings infuscated, the veins piceous.

The two females from which the foregoing description was made were taken in Illinois, one in 1897 at Chicago, and the other on August 16, 1899, at Meredosia, in central Illinois, where the fauna and flora are decidedly southern. This is a very distinct species.

PARNOPES WESTCOTTII, sp. nov.

Male.—Length, 7 mm. Bright metallic green varied with coppery reflections. Rugosely punctured. Covered with silvery pubescence as follows: face as far as anterior ocellus densely; metapleuræ densely; sides of scutellum and of postscutellum densely; tegulæ sparsely; suture between prothorax and metathorax sparsely; posterior portions of abdominal segments sparsely, but more densely at the sides; and distal portions of the legs moderately. A few scattered hairs are present on the occiput, thoracic dorsum, coxæ, and femora. Punctures rather irregularly placed, stronger on thorax than on abdomen, confluent behind the ocelli, where they are radially arranged, the rows diverging anteriorly. Antennæ with the scape piceous and flagellum yellowish. Clypeus emarginate, although not so strongly as in *festitus*, yellowish at apex. Mandibles rufous, metallic green at base and black at tip. Proboscis reaching back to the middle of the first abdominal segment. Thorax green, with bronze tint, especially when viewed from in front. Prothorax with a rather well-marked median impression. Tegulæ broad, yellow, disc darker, sparsely punctured. Between the parapsidal grooves the mesonotum is less brilliant. Scutellum and postscutellum piceous, more strongly punctured than the rest of the thorax. Postscutellum excised to nearly its middle, the sinus rounded at the bottom and with a width of about 70 degrees. Sides of postscutellum slightly rounded outwardly. Abdominal segments blue at anterior margin, varying through green and cupreous to piceous on posterior depressed third. This depressed portion is provided with much smaller and confluen, punctures, and with the white pubescence parted on the median line. Fourth segment with two wide subapical oblique grooves separated posteriorly, beyond which it is fulvous: its apical margin denticulate, with seventeen teeth in upper rows, below which is a series of lighter colored and more pronounced teeth, the apical two of which are almost spiniform. Venter shining, uniformly fulvous. Legs fulvous, excepting the metallic green coxæ and femora. The trochanters, knees, and upper surface of anterior femora are lighter. Wings hyaline, the nervures testaceous.

Female.—Length, 6.5 mm. Differs from male as follows: Color, a stronger green, with less of the cupreous reflections. Face broader. Scape of antennæ with greenish tinge. Clypeus not so evidently emarginate, black. Mandibles black except rufous spot in middle. Proboscis reaching as far as last abdominal segment. Occipital punctures not radially arranged. Sides of postscutellum, straight. Apical depressed bands of abdominal segments much narrower. The third segment (apical) has

narrower, shorter, and more oblique, subapical grooves nearer the tip of the segment, its apex with less of the fulvous color. The upper apical teeth less stronger pronounced than in male, lower ones more pronounced. Venter piceous.

This interesting little species, the smallest North American representative of the genus is described from two specimens, the male, taken in California, during August, by Dr. O. S. Westcott and the female during last June at Galveston, Texas, by ourselves. It is not probable that the two sexes represent different species, for the arrangement of the teeth of the apical abdominal segment is not constant in *Parnopes*. The difference in coloration seems largely due to the age of the specimens. The male specimen seems not to be so fully chitinized as the other.

The nearest congener of this species is *fulvicornis*, from Presidio, Mexico. It is readily distinguished by the tabulated differences. A specimen from Washington, in the collection of the American Entomological Society seems referable to this species.

PSAMMOPHILA Dahlbom.

The genus *Psammophila*, established by Dahlbom, in 1842, is now represented by at least six species from America, north of Mexico; and possibly by another species (*violaceipennis*) which was described by St. Fargeau as American. As the species was from Pennsylvania and has not since been recognized, it seems quite possible that the type was not from America. From his description it is no doubt a *Psammophila* and has been included in the table.

Ammophila collaris Cresson, which has been referred to *Psammophila* does not belong there; it is a true *Ammophila*.

The following dichotomy will serve to separate the species:

Body wholly black 3
Abdomen more or less ferruginous 2
2. Pubescence of thorax wholly black 4
Pubescence of thorax more or less gray or white 5
3. Face and clypeus without silvery pile, body polished ..... 3 *luctuosa* Sm.
Face and clypeus silvery pilose, body more or less silvery pollinose.
4 *argentifrons* Cress.
4. Abdomen entirely ferruginous, mandibles wholly black ..... 1 *valida* Cress.
Abdomen in part black 6
5. Petiole short, not extending beyond posterior trochanters, stout species, 22 mm. ..... 2 *grossa* Cress., ♂.

Petiole much longer, smaller and more slender species 8

6. "Wings black, violaceous, dilated part of petiole, second abdominal segment, and anterior border of third, ferruginous" ..... 7 *violaceipennis* St. Farg.

Wings much lighter, petiole black 7

7. Petiole short, not extending beyond posterior trochanters, stout species.
2 *grossa* Cress., ♀.

Petiole extending far beyond trochanters, slender species.
5 *communis* Cress., ♂, ♀.

8. Third submarginal cell small, barrel-shaped, eyes strongly convergent below.
6 *pacifica*, sp. nov.

Third submarginal not barrel-shaped, eyes only slightly convergent, pubescence often dark..... .....6 *communis* Cress., ♂.

PSAMMOPHILA GROSSA Cresson.

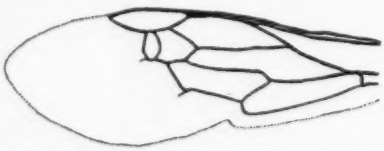
Male.—Length, 22–24 mm. Wholly black, except ferruginous band on mandibles; second abdominal segment below; and narrow bands on third and fourth segments anteriorly above, dilating on the sides and coalescent below; ferruginous; so that second to sixth ventral segments are ferruginous. The dorsal segments also show faint ferruginous bands posteriorly on second to sixth segments, diminishing posteriorly; while in one specimen the black is considerably reduced, until the second and third segments are almost wholly ferruginous. Clypeus and face to considerably above antennæ silvery pubesent. Head, thorax and coxæ bearing long cinereous hairs. Legs white pollinose in certain lights and abdomen slightly so. Clypeus subtruncate, narrowly margined and not dentate; flat below, slightly convex above. Antennæ slightly attenuated toward tip, the apical eight joints each with a sharp carina inwardly. Head very sparsely and rather coarsely punctured. Dorsulum more densely and coarsely punctured. Mesothorax with a short sharp groove anteriorly. Mesopleuræ more densely and coarsely punctate. Metathorax obliquely aciculate on the sides, rugose reticulate dorsally, Legs and abdomen very stout, legs with black spines. Wings light brown, with a violaceous tinge, darker on exterior border. Third submarginal cell oblique, narrowed above. Second submarginal cell receiving the recurrent nervures just before the middle and apex.

Described from two specimens collected at Austin, Texas, during May, 1900.

Although the males differ by their white pubescence from the female described by Cresson, there seems to be no doubt as to their identity, as most specimens of *communis* present the same white pubescence in the males and not in the females. Other specimens of *communis* ♂ collected in Illinois have the pubescence wholly or partially black.

PSAMMOPHILA PACIFICA, sp. nov.

Male.—Length, 12 mm. Black shining, first, second and third abdominal segments bright ferruginous. Clothed with very long pubescence which is white upon the thorax and mixed with black upon the head, especially upon the vertex and face. Head sparsely punctate except around ocelli where it is smooth. Antennæ black, slender, moderately long. Face and clypeus to above antennæ silvery pubescent. Clypeus broadly subtruncate. Mandibles indistinctly annulate with ferruginous. Eyes strongly convergent below, reaching nearly to base of mandibles. Prothorax and mesonotum sparsely coarsely punctured. Pleuræ and sides of metathorax coarsely and densely punctured. Scutellum strongly longitudinally aciculate, the grooves widening out posteriorly to form a row of



FORE-WING OF PSAMMOPHILA PACIFICA.

pits along the posterior margin. Posterior face of metathorax finely transversely aciculate. Coxæ black, clothed with long white hairs which also extend to bases of femora. Tarsi slightly tinged with fuscous, thinly white pollinose. Abdominal petiole black, white pilose anteriorly. Abdomen very slender, first, second and third segments bright ferruginous, remainder shining black. External genital organs fuscous. Their lamellæ with several stiff bristles interiorly and a brush of hairs externally. Anterior wings hyaline, brownish at tip, posterior one hyaline. Marginal cell extending far beyond third submarginal cell, which is less than half as large as the second and barrel-shaped.

Described from a male specimen collected by Miss Rose Patterson at Pacific Grove, California, July 9, 1897.

This species may be readily recognized by the venation of the wings, the strongly convergent eyes, white pubescence and very slender form.

1.	*P. valida* Cress.	21 mm.	Colo.
2.	*P. grossa* Cress.	22–24 mm.	Texas.
3.	*P. luctuosa* Sm.	17 mm.	U. S.
4.	*P. argentifrons* Cress.	13–15 mm.	Colo., N. Mex.
5.	*P. communis* Cress.	12.5–18 mm.	Colo., Ill., N. Mex., Mass.
6.	*P. pacifica*, sp. nov.	12 mm.	Cal.
7.	*P* (?) *violaceipennis* St. Farg.	21 mm.	Philadelphia, Pa.

THE ACCESSORY CHROMOSOME—SEX DETERMINANT?

C. E. McCLUNG.

Part I. Observations and Comparisons.

The peculiar chromatic element discussed under this name in several recent papers is one that gives promise of throwing considerable light upon the nature of the chromosomes. So long as all chromosomes of the nucleus were observed to pass through a cycle of changes apparently identical in each case, there was little chance to gain an insight into their interrelations. With the discovery of the accessory chromosome and the recognition of its true chromosomic character, however, there has been offered an opportunity to draw comparisons and so to formulate conclusions which, in time, are certain to materially increase our knowledge of these most important nuclear structures.

In recognition of this fact, and with the hope of hastening such a desirable end, I have devoted some time to the study of the accessory chromosome and have also encouraged students in my laboratory to direct their attention to it. Much material has been collected, and is still being accumulated, in order that as broad a view of the subject as possible could be obtained.

Since, however, the difficulties involved in securing and preparing material from widely different forms would unduly delay the attainment of any comparative results, I have confined my studies largely to the Orthoptera. This has been done in the belief that more substantial good can be derived from a thorough knowledge of a limited group than from a superficial acquaintance with a wider field. Once the basic principles underlying the cellular phenomena of one group are discovered, their recognition in other forms will be rendered much easier.

As a result of the studies so far pursued, it has been found that individual forms rarely present all the details of a problem equally well. Different species excel in the clearness with which certain points are brought out. A feature obscure in one species will appear distinct enough in another while for the elu-

cidation of other structural peculiarities the relations might be reversed. By studying, therefore, an extensive collection of nearly related forms, it is possible to draw a composite outline of a process which will be found applicable in its main features to all the members of the group concerned.

This fact is taken advantage of in the series of studies upon insect spermatogenesis now being pursued by myself and students. Instead of taking one species and endeavoring immediately to make out the entire series of processes which characterize its spermatogenesis, we have considered restricted questions, and have chosen such species as would offer the best facilities for answering these. Thus, in the case of the accessory chromosome, it has been found advantageous to trace its course through the spermatogonial divisions in *Brachystola*, and through the spermatocyte changes in *Hippiscus*. Probably the spermatid transformations will demand another form for their best exemplification.

The danger involved in such a method is the liability which it offers toward accumulating a series of observations upon exceptional or strongly modified types. This has been guarded against, as far as possible, by verifying the appearances of one form by those manifested in others. While the work thus far done indicates considerable variations in the details of the different processes, it does not seem to suggest any wide deviations from general principles, so that the danger of error in the direction of exceptional instances would not appear to be great.

A recognition of the accessory chromosome as such is of very recent date and the literature upon it is therefore not very extensive. And while its chromosomic character has, in a number of instances, been appreciated and even its participation in the supreme division act of the chromosomes in the metaphase noted, the mere resemblance in general features to the nucleoli has sufficed to caused its inclusion in the group of these questionable bodies. A majority of the references to it will accordingly be found in the literature devoted to a consideration of the nucleoli, and in only a few cases will it be found discussed as a chromosome.

On account of the scantiness of the literature, it has been

thought advisable to give here all the probable references to the accessory chromosome in the language of the different authors. It may be possible thus to unify the conflicting opinions regarding the essential features and to leave the field clear for a discussion of the more involved points. Also, in furtherance of the plan previously mentioned of confining the question to a limited area, only the references to insects will be discussed in detail. By this restriction, however, very little material will be excluded since most of the work has been done upon insect testes.

In the following paragraphs will be found all the references—in the literature at my command—to structures which I regard as possibly identical with the accessory chromosome. These will be given chronologically and finally compared and commented upon.

1. The work of Platner ('86) upon the Lepidoptera unfortunately sheds no light upon the nature of the accessory chromosome in that order of insects. Only casual mention is made of the nucleolus and the references do not enable us to gain much insight into its character. The following quotations embrace the principal references: "An letztern Punkten haben auch die Nucleolen ihre Lage. Diese erscheinen selten in der Einzahl, meist findet man deren zwei. Sie sind von ziemlicher Grösse, färben sich stärker und zeigen eine kugelige Form; doch geben meist die von allen Seiten an sie herantretenden Kernfäden ihnen ein unregelmässiges zackiges Ansehen."

2. "Diese (Elemente) färben sich gleichmässig stark mit Safranin und zeigen nicht nur in ihren Dimensionen sondern auch hinsichtlich ihrer Zahl grosse Schwankungen. Unter ihnen befinden sich auch die Nucleoli."

3. Henking ('90) finds in the spermatogonia of *Pyrrhocoris*, in preparations fixed with picro-acetic acid, a nucleolus which is not apparent in those preserved with Flemming's fluid. It appears rounded and of some size, and retains a yellow color while the chromatin stains dark red with carmine. It is observed occasionally to have divided.

4. Concerning the "nucleolus" of the first spermatocyte, Henking has this to say: "Vor allem auffällig ist es an diesem Zellen dass ein grosser Nucleolus zur Ausbildung gekommen

ist. Derselbe tritt bei den verschiedensten Konservirungsmethoden stets sharf hervor. . . . Erscheint der Nucleolus der jüngsten Hodenzellen bei der genannten Methode farblos, so nimmt er nun begierig Farbe auf, ein Verhalten, welches ganz regelmässig ist und vielfach von den beiderseitigen Nucleolus auf dem gleichen Schnitte beobachtet werden kann."

5. The position of the nucleolus is referred to as follows: "In einer Bucht an der Oberfläche der Chromatinkügelchen liegt der Nucleolus eingesenkt."

6. Regarding the constancy of appearance exhibited by this element, he says: "Allein der Nucleolus hat bei den Veränderungen von Kern und Zelle sein Aussehen nicht gewechselt. Er liegt noch wie zu Anfang als rundlicher Körper dem Rande des Kernes angenähert, . . . Der Nucleolus behalt seine Kugelgestalt unverändert bei, während die Chromosomen gewissermassen Pseudopodien aussenden und sich so zu einem Netz vereinigen. Der Nucleolus bietet seinerseits den Pseudopodien keine Ansatzfläschen und bleibt daher isolirt."

7. Zur Zeit der zusammenballung der Chromatinmassen ist er durch seine beträchtlicher Grösse immer noch leicht zu sehn und seine Kugelform macht ihn kenntlich wenn die Chromosomen durch Zusammenfliessen des Chromatins an Volumen ihn zu überragen beginnen. Wenn dann aber die Auflockerung des centralen Haufens anhebt, tritt eine Verkürtzung der Chromosomen ein, wodurch dieselben ihm immer ähnlicher werden. Schliesslich ist er nicht mehr mit Sicherheit heranzufinden. Dafür das er gänzlich rückgebildet wird habe ich gar keine Andeutung erhalten. Er ist so lange in voller Ausbildung deutlich zu erkennen, als ihn seine characteristiche Gestalt vor einer Verwechselung mit anderen Gebildung schützt. Allerdings müssen wir annehmen das er späterhin eine Einschnürung erfahrt da auf einem definitiven Stadium alle Chromosomen eine gleiche Form besitzen.

8. Having thus traced his "Nucleolus" up to its final disappearance among the group of chromosomes in the first spermatocyte, Henking leaves it and goes on to a consideration of the division of these elements. He notes here the peculiar character and behavior of one "pair" of chromosomes which is noticeable

on account of its deep staining power and inertia during division. This latter is so strong as to cause it to remain undivided in the second spermatocyte mitosis, and so to pass into only one of the two resulting spermatids. It is, therefore, as the "Doppelelement *x*." instead of the "Nucleolus *n*." that we trace its further history.

9. After an extended discussion of the subject, embracing an account of the delayed division of the unusual chromosome of the first spermatocyte mitosis and its later behavior, Henking sums up his conclusions in the following words: "Demnach glaube ich sagen zu dürfen: Bei der letzen Theilung der Spermatocyten wird das Chromatin ungleich getheilt, derart, das die eine Spermatide nur 11 Chromosomen erhält, die andere dagegen ausser den 11 Schwesterchromosomen noch ein ungetheilt bleibendes Chromatinelement."

10. With respect to the further behavior of this latter element, Henking observes that "Nur das isolirt Körperchen hält sich davon entfernt, tritt nicht in so einige Berührung mit den 11 Chromosomen."

11. Under the treatment of the spermatid transformations, Henking discusses the participation of his "isolirt Einzelelement" in the following language: "Von der allgemeinen Vertheilung hält sich nur das isolirt Einzelelement zuruck, welches bei der letzten Halbirung der Spermatocyten ungetheilt in die eine Tochterzellen übergegangen war. Es sind somit die, wenn wir so wollen, bevorzugten Tochterzellen auch jetzt immer noch zu erkennen.

12. His final opinion of the element is expressed in the following quotations: "Ich glaube, ein jeder unbefangene Beobachter wird mit mir diesen runden von dem übrigen Chromatin scharf unterschiedenen Körper für das Kernkörperchen ansehen. Damit ergiebt sich aber die wichtige Thatsache, *dass wir zweierlei Spermatozoen erhalten: die einen besitzen einen Nucleolus, die anderen nicht.*"

13. In the summary of his results, he expresses the same thoughts in slightly different language. He says: "Es sind zwei verschiedenwerthige Arten von normalen Samenfäden vorhanden. Die einen enthalten nur 11 chromatische elemente, die

andern ausser 11 chromatischen Elementen auch noch ein einzelnes zuletzt ungetheilt gebliebenes Chromatinelement, welches wahrscheinlich als Nucleolus anzusehn ist."

14. The investigations of Toyama ('94) upon *Bombyx* and several other Lepidoptera, like those of Platner, do not afford us any very definite idea of the accessory chromosome in this order. Several references to nucleoli are made, however, and these I will quote:

15. "The nucleolus (first spermatocyte), lying either in the chromatin mass or outside of it, persists, as is unusual in skein stages of other animals, till to the end of the skein stage shortly to be described. . . . The nucleolus, however, shows no change from the first resting stage till the present stage, and always consists of small chromatic granules imbedded in a less stainable matrix.

16. "In a still later stage the chromatin granules again commence to separate from one another, and the nucleus again presents the appearance shown in Figs. 31 and 32. In most cases two nucleoli are found in the nucleus of this stage. These gradually migrate toward the periphery of the nucleus facing the center of the cyste (rarely, facing the wall of the cyste) and are finally pushed out into the cytoplasm one after the other through the nuclear wall at this point. Placed in the cytoplasm the nucleoli seem to change their quality, since they now stain differently from what they did when they were in the nucleus. This is shown by the use of Hermann's triple staining, by which the nucleolus in the cytoplasm takes a brownish color, while it colors deep red so long as it is within the nucleus. The further fate of the nucleoli in the cytoplasm is not known.

17. "In this stage I have not found any nucleolus in the 'Kernplatte,' while Henking observed it in a spermatocyte of *Pyrrhocoris apterus*."

18. Wilcox ('95) in his studies upon *Caloptenus femur-rubrum* and *Cicada tibicen* finds peculiar nucleolar structures which will be found described in the following excerpts:

19. "Cytoplasm and achromatic nuclear parts were stained green, the chromosomes, nucleolus, and centrosomes red (safranin and victoria-green). . . . In *some* stages the chromosomes were

stained green, indicating that a chemical change takes place in the chromatic substance. But even in such cases the nucleolus was bright red. . . . By this method (Henneguy's) the chromosomes and nucleoli are stained bright red, the individual chromosomes being sharply outlined. . . . During the stages shown in Figs. 49, 51, 52 (spermatocytes of *Cicada*), there appears to be a chemical change in the constitution of the chromosomes. By the safranin and victoria-green method the chromosomes stain red, though not so deeply as the nucleoli. At later stages the chromosomes assume a green color, while the nucleoli continue to stain red. In still later stages the chromosomes again take the red.

20. "One or often two nucleoli are to be seen (spermatogonia of *Cicada*). . . . The cells of *b* (a spermatocyte cyst) each contain one or two bodies which I consider nucleoli, since they react to the stains quite differently from the chromosomes. . . . The nucleolus then moves to the periphery of the nucleus, and appears meantime to have divided into two portions, one of which passes into the cytoplasm, while the other remains in the nucleus; later, both parts appear outside the nucleus and on diametrically opposite sides of it."

21. With this incomplete account, the nucleolus is left by Wilcox. But in a subsequent paper ('96) he takes up the later history of the element and carries it into the spermatozoon. Two paragraphs will give his conclusions.

22. "The body which appears in the vacuole of the nucleus is rather problematical, both as to its origin and its fate. It appears usually as a rod of deeply staining substance, whose longest axis is in the long axis of the vacuole; but the rod may have the form of a crescent.

23. "The tentative conclusion to which I have come with regard to this body is, that it represents the nucleolar substance of the nucleus of the spermatid, and that it subsequently passes into the mass of chromatin, with which it becomes homogeneously mingled. My evidence for this is as follows: Very soon after the second division of the spermatocytes a body is seen in the nucleus. It (*cres.*) lies at first among the chromatic granules of the nucleus, but is distinguishable from any of the latter by its

greater size and deeper color. Then it comes to lie in the vacuole of the nucleus. At length, what I consider its remains are found for some time faintly discernible in the chromatic mass of the head of the immature spermatozoon. In later stages this body is not to be distinguished from the rest of the chromatic mass. I was at first inclined to believe that this body allied itself with the centrosome to help in forming the neck-body, but was soon convinced that this is not true, because I observed that the two parts of the centrosome and this problematical body exist at the same time in the same spermatid. In Figs. 90, 92, and 106 the body in question is seen in contact with the chromatic mass, and in Figs. 103, 104, it is nearly included in the chromatic crescent. Later, as already indicated, it becomes indistinguishable from the rest of the head of the spermatozoon. Accordingly, I am unable to determine whether or not it forms any definitely limited portion of the head."

24. While Henking was the first to discover the unusual behavior of a "nucleolus" in the spermatocyte which, in all of its manifestations but one, corresponds to an ordinary chromosome, Montgomery ('98) deserves the credit for observing that this body is merely a chromosome of the spermatogonia that pursues a somewhat different course from the others. Unfortunately, however, his interest in nucleolar structure led him to denominate it, with Henking, a nucleolus and it is accordingly under the name "chromatin nucleolus" that we shall trace its history through the spermatocytes of *Euchistus*.

25. The persistence of a color reaction in this element that is characteristic of chromosomes in the metaphase first attracts Montgomery's attention. He states in regard to this that "in each nucleus, from the commencement of the anaphase on, one of the chromosomes still retains the red stain characteristic of all of them in the immediately preceding period, and this particular element is destined to become the chromatin-nucleolus, the metamorphosis of which will be described later."

26. Subsequently he discusses the chromatin nucleolus at some length and from this part of his paper I will quote passages bearing upon its name, staining reaction, form, behavior, final disposition, and function. He says:

27. "To return to the chromatin nucleolus. I give this name in order to express its genetic origin, and to distinguish it from the true nucleolus; it differs also from the "karyosomes" found in many cells, which are nothing more than temporarily thickened portions of the chromatin reticulum. . . . But when the chromosomes have become more or less elongated, all of them stain violet (with increasing intensity of color), except one, which remains red (saffranine), and by strong light may be easily distinguished from the other chromosomes. This one is the chromatin nucleolus, characteristic for the spermatocytes. At least one whole chromosome becomes thus metamorphosed; and it is very probable, judging from my observations, that only one becomes thus changed. This chromatin nucleolus retains in all stages up to the formation of the spermatids its red coloration after the use of Hermann's double stain, and so can be easily distinguished from the true nucleolus as well as from the chromatin of the rest stage and anaphase.

28. "The chromatin nucleolus appears to undergo the same changes of form as do the other chromosomes, up to about the synapsis. Then it ceases to elongate, and in the post synapsis gradually commences to assume a spherical form, which is characteristic for it during the telophase and the rest. When it may first be distinguished in the early anaphase, and also during the synapsis, it lies within the nuclear cavity, not in contact with the nuclear membrane; but at the end of the synapsis it gradually takes up a more peripheral position, so that usually during the synapsis, and always in the telophase and rest, it is closely apposed to the nuclear membrane. . . . But in most cells in the synapsis it occurs in the nuclear cavity apart from the chromosomes. In such cases it is found to be usually rod-shaped, often more or less curved, occasionally even lobular; but so great is its irregularity in form, that in no two cases does it have exactly the same shape. . . . Afterwards it either gradually shortens up into the ultimate spherical form, or first becomes constricted at one or more points on its surface, showing then a more or less beaded appearance, and then, by division at these points, breaks into a number of unequal fragments, each of the latter subsequently rounding off. . . . But it is most probable that at first

only a single one is present, *i. e.*, that only one chromosome becomes changed into a chromatin nucleolus: for in the synapsis, when it may best be distinguished from the chromosomes, I have never seen more than one long chromatin nucleolus. From the synapsis on, the surface of the chromatin nucleolus gradually becomes smooth, so that its process of rounding off may be regarded as a mode of concentration of its substance. It finally becomes homogeneous, quite different from the microsomal chromosomes.

29. "These phenomena show us the remarkable process of the casting off of an entire chromosome, which is itself possibly a mode of chromatin reduction; and in the two spermatocytic divisions we shall find that the chromatin nucleolus does not again become a chromosome. . . . There are only two other thinkable modes of origin of the chromatin nucleolus: 1) that it be extranuclear in origin, or 2) that it be a secretion of the chromatin.

30. "In the spermatocytes of *Harpalus* a chromatin nucleolus has been seen by me, besides the true nucleolus; and judging from the observations of authors on various objects it would seem that such a structure is generally characteristic of spermatocytes. Thus Moore ('95) found in Elasmobranchs about the beginning of the synapsis, 'a curious secondary nucleolus surrounded by a vacuole, which, so far as I can ascertain, is in these fishes characteristic of this change.'

31. "Thus the true nucleolus passes from the periphery toward the center of the nucleus, the chromatin nucleolus in the reverse direction.

32. "The chromatin nucleolus lies now in contact with the nuclear membrane, and is rounded with the exception of that side flattened against the nuclear membrane.

33. "In the early prophases the chromatin nucleolus becomes rounded, but at first retains its central clearer globule. At the loose spireme stage it commences to grow smaller, at the same time losing the central globule. . . . The decrease in size continues until the end of the loose spirem, when a dimension is attained which is approximately uniform for the chromatin nucleoli of all cells; one or more of the smaller bodies, which arose as

fragments of the original chromatin nucleolus, may still be seen in the nucleus, and often up to the monaster stage. At the time when the chromosomes have attained their definitive form, it usually becomes likewise elongated and dumb-bell-shaped; in the majority of cases it appears to assume this form before the nuclear membrane disappears. Thus it looks like a diminutive chromosome among larger ones. As the true chromosomes now stain with saffranine it likewise resembles them in coloration. This peculiar structure acted like a nucleolus in the rest stage, but in the monaster is destined to lie in the equator among the chromosomes, where it also becomes divided in metakinesis, and so terminates in acting like a chromosome, as at the commencement it has been formed from one.

34. "In a few cases, so few that they must be considered abnormal, a whole undivided chromosome passes into a second spermatocyte, but I have met with only two or three such cases. Henking found in *Pyrrhocoris* and later in some other cases, that the second spermatocytes receive an unequal number of chromosomes, *i. e.*, that one of them may frequently if not usually receive a whole undivided chromosome; either *Pyrrhocoris* shows a marked peculiarity in this respect, or else Henking had mistaken either a yolk globule or a chromatin nucleolus for an undivided chromosome. . . . Each second spermatocyte appears as a rule, if not always, to receive a half of the original chromatin nucleolus.

35. "The 7 chromosomes and the chromatin nucleolus gradually become arranged in the equator of the spindle, their axis parallel to the latter, and the plane of their constrictions perpendicular to it. Then follows the metakinesis, with a consequent transverse (reduction) division of all the chromosomes, and apparently in most cases of the chromatin nucleolus, with the result that each daughter cell (spermatid) receives 7 daughter chromosomes and 1 daughter nucleolus."

36. Paulmier ('99) records in his observations that "These interesting bodies (the small chromosomes) were first recognized in the equatorial plate of the spermatogone divisions in the form of two chromatin masses very much smaller than the chromosomes and connected with them by chromatin bands. In the

resting spermatogones they appear as two rather indefinite bodies. staining with the chromatin stains, and apparently not breaking down to the same extent as the rest of the chromatin. During the period of spermatocyte growth they come to view again in the synapsis stage, as a single body. This body has at first an irregular shape, then it elongates, splits longitudinally, and again transversely, thus forming a tetrad in essentially the normal manner, though passing over many of the stages which the other tetrads go through. During these stages it shows a decided difference in its staining reactions, taking at all times a deep black stain with hæmatoxylin, while the rest of the chromatin is scattered irregularly and stains gray. It always lies close against the nuclear membrane. In the first spermatocyte division it lies in the center of the ring of chromosomes, and divides somewhat before the others. The two parts are connected with each other by two threads precisely as are the normal tetrads.

37. "In the second spermatocyte division it goes bodily over into one of the two daughter-nuclei without showing any traces of division, beyond a slight elongation due to the pull of opposing spindle fibers. In a slightly later stage it again shows its difference from the other chromosomes by retreating as far as possible from them. Soon the disintegrating force overtakes it, and it becomes indistinguishable from the others.

38. "I think that we may say without hesitation that this body is not a true nucleolus, a possibility precluded by its different staining reaction, the constancy of its occurrence, and by its division. We find also in addition to it a perfectly normal, true nucleolus in both the resting spermatogones and spermatocytes.

39. "I agree with Henking that it is chromatin, and that the nuclear substance is thus divided unequally. This body is absent in one-half of the spermatozoa which nevertheless, as far as we know, produce normal descendants. I would make the suggestion that it is degenerating chromatin; in other words, that these small chromosomes, or idants (to adopt for the moment Weismann's terminology), contain "ids" which represent somatic characters which belonged to the species in former times, but which characters are disappearing. The "ids" which represented these characters are much slower in disappearing than the characters

themselves, and persist as the two small chromosomes of the spermatogones. These then undergo a pseudo-reduction and form a tetrad which is unable to complete the second spermatocyte division."

40. My own early views concerning the accessory chromosome, as manifested in the Locustidæ, may be found expressed in the following quotations from my first paper ('99) upon the subject:

41. "As it first appears in the spermatogonia of *Xiphidium fasciatum*, there would be no hesitation in calling it a nucleolus except for its unusual situation on the surface of the nuclear vesicle. It is a small, irregularly rounded body, and lies immediately under the nuclear membrane. Before the division figure is established, however, it takes on the form of a thread which becomes 'U'-shaped. Still further contraction ensues, and by the time of the metaphase the thread has become very short and thick and is bent in the middle with an obtuse angle so as to resemble a boomerang. At this time, it may be observed lying at one side of the circle of chromosomes arranged in the equatorial plate, and plainly distinguishable from them by reason of its greater length. From the pole the chromatin appears as a broad, fenestrated plate, and the accessory chromosome is indistinguishable from the ordinary ones. Because of the rapidity of the division none of the anaphases are to be seen, but in the telophases the ordinary chromosomes of the cell may be seen grouped in the typical manner at the two ends of the spindle, while extending down towards the equatorial plate from each mass is a half of the boomerang-shaped body which has been divided longitudinally in the same manner as the ordinary chromosomes.

42. "In the resting stage of the spermatocyte that succeeds the appearance just described, the accessory chromosome again appears as it did in the resting stage of the spermatogonia, and would easily be taken for an ordinary nucleolus. Soon, however, it commences to assume a thread-like form which finally results in the production of a long 'U'-shaped body, a form that is retained during the greater part of the spireme stage. In this condition it lies at the surface of the vesicle and stains in its usual intense manner. Concurrently with the formation of the 'rings'

from the spireme thread, it commences to shorten and grows into the form of a horseshoe, and is finally to be distinguished from the chromatic rings only by its deeper staining quality and by the smoothness of its outline. In the formation of the mitotic figure of the first spermatocyte division, it assumes its position on the outside of the group of chromosomes as it did in the spermatogonial division, and again has the boomerang shape that marked its appearance in the early figures. When the chromatin separates and moves to the two poles, the accessory chromosome divides longitudinally and presents the appearance of two horseshoes with their rounded ends in contact. In the second spermatocyte division apparently the same process is followed.

43. "The recently formed spermatids possess a nucleus in which the ordinary chromatin is extremely scant and very weak in staining power, while the accessory chromosome shows as prominently as ever and stains in the same uniform manner. It is not easy to trace out the part that the different elements of the nucleus take in the formation of the spermatozoon, but in the light of present knowledge it appears as if the accessory chromosome was prominently concerned in the formation of the head.

44. "In seeking to point out the features that characterize this peculiar nuclear element, perhaps the most striking thing to be noticed, is the almost uniform staining power exhibited.

45. "Thus there seems to be no reason to suppose that the accessory chromosome of *Xiphidium* arises by the direct transformation of one of the ordinary ones, although such a change may be possible. This does not argue against the chromatic origin of the body, however, for it is almost certainly modified chromatin, but in *Xiphidium* it arises during the resting stage and may represent derivative substance from one or all the chromosomes."

46. A later, more detailed, study of various members of the Acrididæ ('00) gave me a much better insight into the nature of the accessory chromosome and the result of my observations upon its behavior during the spermatocyte divisions are summarized in the following words:

47 "As a result of the last spermatogonial division, the much reduced daughter cells are each provided with the somatic num-

ber of chromosomes. All but one of these rapidly disintegrate and from their substance produce the spireme of the first spermatocyte. One persists in its original form and, assuming a peripheral position, continues to stain as does a chromosome of the metaphase. During metakinesis it is divided like other chromosomes. This is the accessory chromosome.

48. "The accessory chromosome is a constant and important element of the germ cell. It arises, in the Acrididæ, from a spermatogonial chromosome, and from that time forward maintains a separate and distinct existence. During the prophases, when occur the profound changes that result in the production of a nucleus with only half the ordinary number of chromosomes, this structure stands aloof and self-contained. With the establishment of the mitotic figure of the first spermatocyte, however, it takes its place with the other chromatic elements and becomes indistinguishable from them henceforth until the spermatids are formed. Here it again becomes distinct and conspicuous."

49. Up to the present time, we have learned nothing of the real origin of the accessory chromosome. That it is one of the spermatogonial chromosomes that passes over into the spermatocytes without taking part in the formation of the spireme is pretty well established; but as to the manner of its appearance in the spermatogonia, we have no knowledge. Sutton ('00) is the only one who has traced the history of the element at all fully in the spermatogonia. Concerning its first appearance and its subsequent history in this generation of sex cells he says, "In *Brachystola*, the accessory chromosome appears probably in the first, and certainly in the third, secondary spermatogonial division, and goes through precisely the same changes in each cycle up to the last.

50. "It may occasionally be distinguished from the other chromosomes in the metaphase and anaphases by its granularity and greater length, though it always divides like the others, and in the actual process of division, as a rule, is indistinguishable from them. In the telophase it constructs its own membrane just as the others do, but soon becomes sharply contrasted with them by the deposition of its chromatin in a diffused condition upon the inner surface of its vesicle (vesicular chromosome), and

also by the fact that from this point to the following metaphase the cavity of its vesicle remains distinct from that formed by all the others. In these stages the vesicle of the accessory chromosome may lie on any portion of the nuclear membrane proper; in some cases occupying a position between the 'fingers' or sacculations of it (Fig. 34). This vesicular stage is of comparatively long duration, and is followed by a receding of the chromomeres from the membrane to form a chromatic rod, first loose, rough, and granular, but gradually growing more slender, and compact, and often becoming twisted (Fig. 16). It also betrays a longitudinal split at a stage later than that at which a similar occurrence is observable in the ordinary chromosomes.

51. "From the middle prophases to the telophases, its conduct is so similar to that of the other chromosomes that it would hardly be an error to speak of the cells of these stages as having two nuclei, one having a single chromosome and the other a large number, the small nucleus always lagging slightly behind the large one. This lagging of the accessory chromosome is nicely shown in Fig. 21, already alluded to, where in a stage just before the metaphase the nuclear membrane is seen to be dissolved while that of the body in question is still intact.

52. "In the last or transformation division of the secondary spermatogonia, some differences are noticeable in the behavior of the element under consideration. The vesicular stage seems to be of slightly longer duration, and while, after its close, the same condensation of the chromatin takes place, no longitudinal split appears until the late prophases or 'ring stage' of the spermatocyte; and in the course of the earlier prophases of the growth period its vesicle gradually becomes fused with the nuclear membrane, its outer half completing the smooth contour of the latter, while its inner portion projects into the nuclear cavity. In this stage it has the appearance of an irregular vesicle filled with a homogeneous, darkly staining liquid or semi-liquid body, suspended within the membrane of the nucleus. Later it again becomes granular, and in the first spermatocyte division divides as it did in the spermatogonia.

53. "The resting stage of this element, as shown by its staining violet with Flemming's three-color stain, is what I have called

the vesicular stage, and this only, since at all other times it stains a bright red. The absence of the formation of a spireme at any stage in the development of this element is paralleled by Henking's description of the normal process in all the chromosomes in the spermatogonia of *Pyrrhocoris*.

54. "Perhaps the most important thing to be gained at present from the knowledge of the behavior of the accessory chromosome in *Brachystola* is the light which it throws upon the question of the individuality of the chromosomes. In the first place, the fact that it is a true chromosome, though different from the others, is shown by its staining reactions and by the parallelism between its development in the spermatogonia and that of its more generally recognized fellows. Although it shows a tendency to lag behind the other chromatic bodies, the only radical difference between the two is the absence of the loose spireme in the accessory, and this is paralleled, as shown above, by the normal process of all the chromosomes of *Pyrrhocoris*, according to the statement of Henking. The apparent radical difference in the case of the vesicular stage is, in reality, only a matter of degree, for it frequently happens that the ordinary chromosomes, in going into the diffused condition, leave a very appreciable hollow in their centers (Fig. 30). Apparently there is, for some reason, a necessity that the chromatic granules of the accessory come into closer relation with the cytoplasm than those of its mates, and the result is their deposition upon the vesicle itself—this vesiculation being really a substitute for the loose spireme so conspicuously lacking.

55. "Now, if it be admitted that the body *is* a chromosome, inspection quickly shows us that it maintains throughout the spermatogonial divisions, as well as in those that follow, an indubitable independence, being enclosed, in all stages except those of actual division, in its own individual membrane. Having, then, one of the chromosomes which preserves its individuality in this way, and seeing the other chromosomes enclosed for a part of their development in similar individual vesicles, which only become intercommunicating by absorption of a part of their walls, have we not a right to suppose that at one time they too enjoyed the same independence as their more exclusive mate? In other

words, have we not a right to suppose that their phylogeny is paralleled by their ontogeny? If this be granted, then we have at least more ground for belief in the individuality of the chromosomes than if we had never known of a time when they were of necessity Independent."

56. Of interest in connection with the question of the general distribution of the accessory chromosome are the observations of Miss Wallace ('00) upon the male germ cells of the spiders. The general facts of the case are found in the following quotations from her preliminary paper: "In this spider the peculiar chromosome is conspicuous in the late spermatogonic stages, and in the prophase of the first spermatocyte, its peripheral position in the nucleus making it easy to observe. Its origin in the spermatogonia has not yet been traced, but a gradual change of form has been made out in the early stages and, suffice it to say here, that in all of them it appears to be double.

57. "In the monaster of the first spermatocyte the accessory chromosomes are easily distinguished from the others by their sharpness of outline, slightly greater affinity for staining reagents and above all by their eccentricity of position. They are always found on the periphery of the spindle and often near the periphery of the cell. It is a curious fact that in the majority of cases both of these elements are found nearer one pole than the other."

58. Regarding the unequal distribution of the accessory chromosome to the spermatozoa, she is unable to speak definitely. Her observations on this point are stated in the following words: "In the spider the position of the two chromosomes nearer one pole gives the impression that this unequal distribution occurs in the first spermatocyte division. One thing, however, opposes this interpretation and that is that in the monaster of the second spermatocyte division two elements are again found in eccentric position but of half the size of those found in the preceding cell-division. The position nearer one pole might mean merely delayed distribution but there is not yet at hand sufficient data from which to draw a conclusion."

59. In the latest edition of his work upon the cell, Wilson ('00) takes cognizance of the investigations being pursued by insect

spermatologists and practically adopts Paulmier's views concerning the accessory chromosome. We find in his summary that "A comparison of the foregoing results indicates that the small tetrad (dyad) corresponds to the extra chromosome observed by Henking in *Pyrrhocoris*, and perhaps also to the 'accessory chromosome' of *Xiphidium*. Whether it corresponds to the 'chromatin nucleolus' of *Pentatoma* is not yet clear. The most remarkable of these strange phenomena is the formation of the small tetrad, which seems to be a non-essential element, since it does not contribute to all the spermatozoa. Paulmier is inclined to ascribe to it a vestigial significance, regarding it as a degenerating chromosomes which has lost its functional value, though still undergoing in some measure its original morphological transformation; in this connection it should be pointed out that the spermatocyte nucleolus, from which it seems to be derived, is represented in the spermatogonia by *two* such nucleoli, just as the single small tetrad is represented by two small chromosomes in the spermatogonia mitoses. The real meaning of the phenomena is, however, wholly conjectural."

Because of the greater prominence of the element in the spermatocytes, observations upon its occurrence and changes have been more numerous and accurate than in the case of the spermatogonia. During the prophase of the first spermatocyte, particularly, the appearance of the element is so striking as to render its oversight impossible. Regarding the main features distinguishing it, there is a convincing agreement in all the published reports and these speak further for the morphological exclusiveness so plainly manifested by the element in the spermatogonia. There are some slight discrepancies in the accounts of its very early appearance but concerning the later stages there appears to be no confusion.

From the Hemiptera (¶ 27) and the Orthoptera (¶ 47), we learn that the chromosomes of the last secondary spermatogonia, with the exception of one, break down rapidly into their constituent chromomeres and that these enter at once into the formation of a spireme—at first thin and fine but later coarse and granular. The exception to this process is the accessory chromosome. It is, in the beginning, somewhat irregular in outline

but quickly condenses its substance and becomes homogeneous, transparent, and sharply outlined. It early assumes a position upon the periphery of the forming nuclear vesicle and maintains this during all the prophase. Throughout this period, and the remaining stages of its existence, it persistently stains according to the reaction exhibited by chromosomes of the metaphase. It is thus, in both its chemical and physical properties, strikingly different from the remainder of the chromatin. It may be said, therefore, that it is a chromosome of the previous generation (*i. e.*, one formed from the spireme of that generation) which exists as such while its fellows pass through the prophase of another mitosis.

So far as the first spermatocytes are concerned, this is the only point at which the accessory chromosome differs from the others. When metakinesis occurs, all the chromatin elements divide at the same time and in the same way. The participation of the accessory chromosome in this act is generally conceded (¶ 33, 36, 47), but the exact process has not yet been observed because no form has been studied where the element stands out conspicuously enough to be noted. From the nature of the element, however, the only reasonable thing to expect would be that it should divide as it has done in all previous mitoses, *i. e.*, longitudinally.

With regard to the action of the accessory chromosome in the second spermatocyte mitosis, there are two opinions. One is that it takes part with the ordinary chromosome in the act of division (¶ 34), the other that it passes undivided into only one of the two resulting cells (¶ 9, 12, 13, 37). Examination of the literature shows that the weight of evidence, so far as observations are concerned, supports the latter view. In addition to this, the fact that it differs from the other chromosomes must be taken into account. When it divides in the first spermatocyte it has finished the entire act of separation begun for it in the prophase where it was formed. It has no need, therefore, to divide in the second mitosis where the other chromosomes complete the separation of the chromatids formed in the prophase of the first spermatocyte. Thus, when the origin of the element is taken into consideration, a phenomenon at first apparently inexplicable

according to the usual laws governing cellular activities is seen to be in strict accord with them. The variation from normal conditions is, accordingly, manifested by those chromosomes which emerge from the spireme of the first spermatocyte instead of by the one which fails to enter into it.

At the completion of the second spermatocyte mitosis the apportionment of the chromatin to the germinal elements is accomplished and the further changes in the spermatid would seem to indicate that there is no longer the necessity for the chromosomes to maintain their separate identities. This, because the chromatin first becomes diffuse and later condensed so as to form the head of the spermatozoon where there is no distinction of parts. In the spermatid where the accessory chromosome is present, there is no apparent difference in the behavior of the nuclear elements. The accessory chromosome runs the course of the ordinary chromosome and sooner or later becomes indistinguishable in the homogeneous mass of the spermatozoon head.

It is, therefore, impossible to trace the location of the accessory chromosome to any portion of this nuclear mass. The fact is apparent enough, however, that it does remain a part of the nuclear contribution to the mature element (¶ 12, 13, 23, 37, 43) and does not go to form a part of the archoplasmic derivations as Wilcox was at first inclined to believe (¶ 23). The merging of the chromatin elements into one mass makes it impossible to go further in the investigation of the accessory chromosome at this point. We can hope to know more about it only by learning its part in fertilization of the egg. As a result of the action of the accessory chromosome in one maturation mitosis, this fact, at any rate must be apparent, viz: that there are two kinds of spermatozoa; those *with* the accessory chromosome and those *without*. Beyond this is speculation only, but with accumulated observations on many forms it may not be long until we are able to reach a definite conclusion regarding the exact function of this well defined element.

From the different observations, I hope (1) to bring out the essential features which characterize the accessory chromosome, (2) to show the extreme probability of its universal occurrence among insects, (3) to outline its history in the different cell gen-

erations of the testis, and (4) to suggest a theory in explanation of its function.

Confirmation of the statement that most references to the accessory chromosome would be found under discussions of nucleolar structures has just been given in the quotations from various papers. When a reason is sought for the classification of such a purely chromosomic element with this heterogeneous group of bodies it is difficult to find any that is sufficient. Interest in other problems has, perhaps, induced investigators to concentrate their attention elsewhere and as a result the accessory chromosome has been assigned relationships entirely foreign to its true nature.

I have, in previous papers ('99, '00), given my reasons for regarding it as a chromosome, so that I shall not have to go much into detail on this point. It would seem sufficient to show that the element *is* a chromosome of the spermatogonia (¶ 27, 41, 47, 49–53) and that it divides in a subsequent spermatocyte mitosis as a chromosome (¶ 33, 36, 42, 47, 48) in order to insure its classification as such. Yet in full recognition of these conditions Montgomery (¶ 27) calls it a nucleolus and insists (¶ 29) that it never again (*i. e.*, in the spermatocytes) becomes a chromosome. While Henking fails to detect the origin of his "nucleolus" from a chromosome of the spermatogonia he recognizes its chromatic character and its participation in the act of metakinesis and even occasionally calls it a "chromatinelement" (¶ 13).

Wilcox (¶ 19) is very careful to note the staining reaction of the nucleolus and that of the chromosomes. This he finds to be identical except in certain stages, and in these, it is the chromosomes proper that weaken in their affinity for the basic anilines while the "nucleolus" consistently reacts to them with the true chromatin reaction. But in considering the "nucleoli" of the spermatocytes (¶ 20) he regards them as such because "they react to the stains quite differently from the chromosomes." Just in what respect this difference lies is not quite manifest from the text, but it is apparently in being uniform in staining instead of variable.

In his early paper Wilcox ('95) does not trace the final history

of the element so carefully as he does in a later one ('96). Here, even after more extended investigation, he is uncertain "both as to its origin and its fate" but inclines to the belief that it is "nucleolar substance" despite the fact that it does become "homogeneously mingled" with the chromatin. As will be noted (¶ 23), he was at first inclined to consider it related in some way to the centrosome, but as a result of more careful study decided that this was a mistake.

In addition to noting the obvious staining reaction and peripheral position of the element in the prophase of the spermatocyte, Paulmier also observed its behavior in the spermatogonial divisions (¶ 36) and subsequently in the different phases of the spermatocytes (¶ 37). As a result of a recognition of its very apparent chromosomic character he agrees with me in calling it a chromosome, but prefers to speak of it as the "small chromosome." I have already pointed out in a previous paper ('00)[1] my reasons for regarding this as a misnomer on account of its usually being larger than the other chromosomes so need not again refer to the subject. Thus, we have as a result of the latest and apparently most accurate work upon the Hemiptera, the history of a small chromosome that puts it in almost complete agreement with the behavior of the accessory chromosome in the Orthoptera.

From the preceding statements of different investigators, it will be apparent, I think, that there is in the spermatocytes of all insects so far studied an unusual nuclear element which is characterized (1) by a remarkable uniformity in staining power, similar to that exhibited by chromosomes in the metaphase; (2) by a continuous peripheral position during the spireme stage, at least; (3) by an isolation from the chromatin reticulum and nonparticipation in its changes; and (4) by fission during metakinesis after the manner of chromosomes. In addition to these features which have generally been recognized, there are others which material of exceptionally good character has rendered apparent to several observers. Of these I wish to speak later but desire here only

[1] ¶ 3, p. 85; latter part of ¶ 3, p. 86; and ¶ 5, p. 89; were attached to the proof as footnotes but were included by the printer in the body of the article. It thus happens that on page 89 a reference to Paulmier's last paper ('99) appears before a criticism of his earlier one ('98).

to show the great probability of the general distribution of the structure among insects.

The published reports of almost all investigators certainly speak very strongly in favor of such a supposition, but in order to assure myself by personal observation that views of such a character were correct, I examined representatives of the different families of the Orthoptera and became convinced that I was right so far. Later, material from the Hemiptera, Neuroptera, Coleoptera, and Lepidoptera was examined only to confirm the opinion that the element in question is a constant character of the insect testicular cells. The recent work of Miss Wallace upon the spider ('00) would seem to indicate that there is no doubt of its presence in the Arachnids, and with its determination in this class the probability of its general occurrence in the Arthropods is largely increased. I may also say in passing that some hurried examinations of vertebrate spermatocytes lead me to believe that the accessory chromosome is likewise present here.

If it be conceded that we are dealing with a common element of the sperm-forming cells, it must also be admitted that there exist extensive variations in its appearance and manifestations. To do this, however, is no more than to concede what is known to be true of all other chromosomes so that such an admission can in no way impair the standing of the accessory chromosome as a constant and important nuclear element. It will therefore be understood that the outline history of its behavior in the different cell generations, which I intend to give, will apply only in general particulars. Its purpose is more to show the importance of the element than to postulate a type.

In the spermatogonia, our knowledge of the accessory chromosome is due almost entirely to Paulmier and to Sutton. The former author notes in a general way (¶ 36) that in the metaphase of the early cell generations there are present two smaller chromatin bodies which, during the breaking down of the spermatogonial chromosomes to form the spireme, do not suffer any very extensive dissolution. Later these appear as a single body in the prophase of the spermatocyte. Montgomery makes no mention of their behavior earlier than the telophase of the last

spermatogonial division but agrees with Paulmier's view concerning their later changes.

Sutton, on the contrary, has devoted his entire attention to the spermatogonial divisions and has given us a fairly complete account of the changes taking place in *Brachystola* (¶ 49–55).

It is probable that this object presents an extreme view of the accessory chromosome. From an inspection of Sutton's preparations and a comparison with other forms, however, I am strongly inclined to the behalf that it is merely a very marked example of a normal process. We may, therefore, take the course followed by the accessory chromosome in the spermatogonia of *Brachystola* as representative since it is all that we have, at present, to base our knowledge upon.

Even with this we have no hint, as to the real origin of this problematical body—a point of great importance. It is hoped that more extensive collections of material will render it possible to learn something of this, but the problem is one of considerable difficulty owing to the compact nature of the early cells and their apparent irregularity in arrangement. A favorable object may be found, however, in which the structure will stand out clearly enough to be accurately studied, and in this event we may discover the conditions determining the setting apart of this one chromosome from all the others.

Sutton was first able to distinguish the accessory chromosome in cysts of eight or sixteen cells. Here, as in the spermatocytes, it seems to be removed as far as possible from the influence of the ordinary chromatin. This is accomplished by an enclosure in a separate vesicle which, as Sutton observes, may almost be regarded as a separate nucleus (¶ 51). A distinct existence is maintained during all the stages when a possible exchange of material between the accessory chromosome and the other chromosomes might be accomplished. Only after the chromosomes are definitely established as independent bodies are the barriers removed and then only long enough to permit the act of metakinesis to take place.

During the period intervening between the acts of division, the conduct of the accessory chromosome parallels that of the nucleus containing the remainder of the chromatin (¶ 50). A

vesicle is formed, the chromatic substance is deposited upon its wall in intimate relation with the cytoplasm, a concentration ensues and a definite chromosome is produced.

These changes, it seems to me, are easily explainable if we regard the conditions under which the processes operate. The divisions of the spermatogonia are rapid and continuous and every factor concerned is subordinated to filling the follicles with spermatogonia as quickly as possible. This, of course, requires a rapid increase in amount of chromatin including that of the accessory chromosome, and so the anabolic processes are facilitated by bringing the chromatin into a position where it can best derive its nourishment from the cytoplasm. The vesiculation of the accessory chromosome is merely an incident, the isolation of the element being the end sought.

Throughout the divisions of the secondary spermatogonia this process continues until the accessory chromosome of, let us say, the primary spermatogonium has been apportioned to each of the many cells that are now ready to transform into spermatocytes, and during this time it has practically been as independent as if it were the chromatin of a separate nucleus.

Part II. Theoretical Considerations.

In seeking an explanation for the unusual phenomena connected with the history of the accessory chromosome in the male germ cells, it is most natural to surmise the existence of a phylogenetic significance. In the spermatagonia what amounts to practically two nuclei in each cell is strongly suggestive, in mere general features, of the appearances manifested in the Protozoa where both macro- and micronuclei are present. The accessory chromosome might be homologized with the micronucleus which serves as a medium of exchange between the organisms during the act of fertilization, but it would be extremely difficult to trace any parallelism between the macronucleus and the real chromosomic vesicle of the spermatogonia. I do not, therefore, believe that we can look in this direction for an explanation of the peculiar character exhibited by the accessory chromosome.

Nor do I believe that there is the least basis for Paulmier's theory that the structure is a degenerating chromosome. There

are many facts which argue against it. Of these I should like to speak in some detail, since the theory they controvert is the only one yet advanced upon the proper basis that the element is a chromosome and not a mere nucleolus.

Paulmier considers the element a chromosome in the process of disappearing from the species and reaches this conclusion after observing that in the last spermatocyte mitosis it fails to divide and is thus unequally apportioned to the resulting spermatozoa. Before considering a theory based upon so unusual a phenomenon as this, it would be well to make certain that it is an actual and common occurrence. That it is must be granted, I think, after the work of Henking, Paulmier, and myself, upon so many different forms, has shown it to be of such wide distribution. Granting this, then, there remains to be examined the validity of the assumption that the act presages the final extinction of an element.

In combating the suggestion of Paulmier, I shall make use of the evidence offered in the different cell generations, commencing with the spermatogonia. Every instance, according to my interpretation, shows facts strongly incompatible with this author's view. I would suggest in this connection that the extreme importance of the structure is unmistakably manifested by its course in the spermatogonial divisions. What could more strongly emphasize the special importance of an element than to have it set apart in a separate vesicle while its fellows are provided with one common investment? Morphologically, this is simply raising the accessory chromosome to the rank of a nucleus coordinate with the one commonly present in a cell. Its careful and uniform division during the mitoses of all the spermatogonia suggests anything but an unimportant structure. Had we no further refutation of the degeneration theory than that afforded by the spermatogonia, it would, I think, be sufficient. The evidence of the other cell generations, however, strengthens this position and is well deserving of attention.

From the spermatogonia, each spermatocyte receives one chromosome (the accessory) which through all the subsequent stages exists as a chromosome and never suffers extensive disturbance of its chromomeres either for the purpose of metabolic activi-

ties or for the possible exchange of mutual influence with the other chromosomes. Montgomery assigns the origin of the accessory chromosome to a single spermatogonial chromosome, in the Hemiptera, and I have clearly traced it to the same source in the Orthoptera. It must be regarded, therefore, as a *single* element and as the possessor of *two* chromatids, after its longitudinal division, and so differs from the other elements which are constituted of *four* chromatids. This is a matter of considerable importance as will appear when we consider the division of the spermatocytes.

The period which witnesses a breaking down of the spermatogonial chromosomes and the construction of a thin chromatin thread from their chromomeres is marked by changes just the reverse on the part of the accessory chromosome. It enters the prophase as a definite body with a staining reaction that is constant and marked. These characters it maintains until it becomes indistinguishable in the spermatid.

Meanwhile the other chromosomes of the spermatogonia are lost in the substance of the spireme, in which condition their chromomeres exist in relations far removed from those prevailing in the component individual chromosome. This spireme stage is one of extreme importance to the structures it involves. In many cases, as has frequently been pointed out, it is a phase occupying relatively, and sometimes actually, a long period of time. During its continuance, profound changes take place in the nucleus as a result of which the chromatin emerges in the form of chromosomes the like of which we are unable to find in any other cells of the body. Instead of having two chromatids at the time of the metaphase, each of these has four. Instead of being as numerous as those of the spermatogonia, there are but half as many. Throughout all the time involved in the production of these fundamental differences, the accessory chromosome has existed quite apart from the field of mutual influence in which the other chromosomes operate. It is thus apparent that it has its characters fixed, not in the generation which witnesses its division but in the previous one. In other words it is a spermatogonial chromosome which divides in the spermatocyte mitosis.

From this it will be apparent enough why there is an undivided chromatin element in one spermatocyte mitosis. It is but a single chromatid and so cannot be separated into halves. If, therefore, Paulmier bases his theory upon this phenomenon (as it appears he does) it would seem that he has a very insufficient foundation for it. True it is that he considers the element a tetrad and so would be more justified in his conception, but the evidence he brings forward in the proof of this is not convincing. Montgomery clearly recognizes its unit character in other members of the Hemiptera, Sutton traces it through the spermatogonia of the Orthoptera as a single chromosome, and my own observations are positive as to its valence. The weight of evidence is therefore strongly against Paulmier on this point—the essential one in his theory. Moreover, I might point out that the division of the elements has already been accomplished in the prophase of the first spermatocyte and only their separation remains for a succeeding metaphase. The active agents here are the archoplasmic fibers, so that failure to act in unison with the other cell structures would reflect upon their vigor rather than upon that of the chromosomes.

Again, the great regularity of the divisions by means of which exactly one half of the spermatozoa are unprovided with the element would seriously weaken any assumption of degeneration. If the usual course of degenerating structures were followed, it would demand great irregularity and uncertainty in the occurrence of the unequal division, whereby varying numbers of the spermatozoa would be marked by the presence of the undivided element. In none of the forms studied is there any suggestion of an indeterminate and indefinite division, and in the absence of this, Paulmier's theory loses its strongest support.

It is also pertinent to ask whether the dropping of specific characters would take place by the elimination of an *entire chromosome*, if so whether this would occur in the germ cells, and, if in the germ cells, why in the last generation?

In view of all the objections advanced, I believe it would be impossible for Paulmier's hypothesis to maintain its ground without the support of numerous others equally difficult to base upon observed facts. It will be necessary on this account to

look elsewhere for an explanation of the various phenomena involved in the problem. I shall therefore venture to advance a theory which has been suggested to me by a careful study of the structure in various species in the hope that an early elimination of the improbable factors of the question will bring us closer to the true explanation.

In offering a theory to account for the function of the accessory chromosome, I do so with considerable reluctance, for I realize how little real general knowledge we have of this structure. It seems to me, however, that something is necessary to concentrate the interest of spermatologists upon the fundamental character of this most suggestive chromatin element, and I know no better way of aiding in this than by publishing the working hypothesis with which I have attacked the problem.

This has led me into the field of theories concerning sex and its determination, but I have tried to avoid any more extensive discussion than is necessary to outline, in a preliminary way, the opinion I hold concerning the meaning of the accessory chromosome. Even with this reservation I have nevertheless been obliged to go further afield than I should desire with our present knowledge as a guide. I can only hope that my excursions may accomplish a measure of the purpose for which they were undertaken.

Briefly stated, then, my conception of the function exercised by the accessory chromosome is that it is the bearer of those qualities which pertain to the male organism, primary among which is the faculty of producing sex cells that have the form of spermatozoa. I have been led to this belief by the favorable response which the element makes to the theoretical requirements conceivably inherent in any structure which might function as a sex determinant.

These requirements, I should consider, are that: (*a*) The element should be chromosomic in character and subject to the laws governing the action of such structures. (*b*) Since it is to determine whether the germ cells are to grow into the passive, yolk-laden ova or into the minute motile spermatozoa, it should be present in all the forming cells until they are definitely established in the cycle of their development. (*c*) As the sexes exist

normally in about equal proportions, it should be present in half the mature germ cells of the sex that bears it. (*d*) Such disposition of the element in the two forms of germ cells, paternal and maternal, should be made as to admit of the readiest response to the demands of environment regarding the proportion of the sexes. (*e*) It should show variations in structure in accordance with the variations of sex potentiality observable in different species. (*f*) In parthenogenesis its function would be assumed by the elements of a certain polar body. It is conceivable, in this regard, that another form of polar body might function as the non-determinant bearing germ cell.

(*a*) If we accept the theory that the chromatin is the bearer of hereditary qualities, there could be little doubt regarding the necessary chromosomic character of a sex determinant. Sex being an elementary characteristic of protoplasm, it would be firmly established in the hereditary basis along with metabolic activity, irritability, etc., and if any argument were needed at all it would be a general one, not concerned immediately with the question under discussion, but with the broader one suggested. It will therefore be assumed that the chromatin is this basis. This being true, it will only be necessary to point out that the work of a majority of investigators definitely proves that the accessory chromosome *is* a chromosome, and its standing in this respect is established.

(*b*) With regard to what would theoretically be required of a chromosome whose function should be the determination of sex, it is probable that almost every investigator would hold an opinion differing in some respects from those entertained by others. What I can suggest in this connection will therefore be merely tentative and an expression of my own views. One thing, however, would seem to be necessary; *i. e.*, that the determinant should exist in the cells until they are definitely established as elements of either an ovary or of a testis.

If it be that the production of male elements is a sign of katabolic conditions, or, in other words, of those that make a greater demand of energy expenditure upon the developing cell, then it would seem most natural that the determinant should be for the purpose of carrying the transformation beyond the production of

ova to spermatozoa. It would therefore be a necessary content of the cells until they had passed through the stages of development beyond that at which they might pause and become laden with yolk or, in other ways, postpone the period of maturation. It is conceivable that the production of four functional cells from one spermatogonium would call for the employment of more energy than would the formation of one functional egg from an oögonium, especially since many cells contribute their substance or support in the upbuilding of the egg.

Accordingly, it would be most reasonable to expect the presence of the determinant in the latest possible stage consistent with its equal distribution to half the spermatozoa. This we find to be the case with the accessory chromosome which regularly occurs in all the cell generations up to the last and is only withheld, finally, from half of the spermatids. By its consistent course in this respect, the accessory chromosome plainly manifests its intimate influence upon the germ cells of which it is a part, and most strongly suggests a relation to sex determination. It may further be pointed out in reference to this relation, that during the multiplied spermatogonial divisions, the accessory chromosome exhibits a somewhat distant attitude toward the remainder of the chromatin, and it is only at the time of the definitive spermatocyte divisions that it comes to be an intimate member of the cell nucleus. In what manner it is borne from the fertilized egg to the testis of the embryo we do not know, and, lacking this knowledge, are placed at a considerable disadvantage for a proper appreciation of its real character.

(*c*) A most significant fact, and one upon which almost all investigators are united in opinion, is that the element is apportioned to but one half of the spermatozoa. Assuming it to be true that the chromatin is the important part of the cell in the matter of heredity, then it follows that we have two kinds of spermatozoa that differ from each other in a vital matter. We expect, therefore, to find in the offspring two sorts of individuals in approximately equal numbers, under normal conditions, that exhibit marked differences in structure. A careful consideration will suggest that nothing but sexual characters thus divides the members of a species into two well-defined groups, and we are

logically forced to the conclusion that the peculiar chromosome has some bearing upon this arrangement.

I must here also point out a fact that does not seem to have the recognition it deserves; viz, that if there is a cross division of the chromosomes in the maturation mitoses there must be two kinds of spermatozoa regardless of the presence of the accessory chromosome. It is thus possible that even in the absence of any specialized element a preponderant maleness would attach to one half the spermatozoa, due to the "qualitative" division of the tetrads.[1]

(*d*) As I elsewhere suggest, it is most appropriate that the sex determinant should have its locus in the spermatozoa. These elements are most commonly freed from any close relation to the parent organism at maturity, and thus lose the opportunity to receive from it any bias toward the production of an unusual proportion of the one sex or the other as environmental conditions might require. It is otherwise with the ova. They are usually retained by the maternal organism in such intimate relation to it that surrounding conditions might easily imprint their demands upon them. Even up to the time of fertilization the female elements are so placed as to react readily to stimuli from the mother. Here they are approached by the wandering male elements from which they may choose—if we may use such a term for what is probably chemical attraction—either the spermatozoa containing the accessory chromosome or those from which it is absent. In the female element, therefore, as in the female organism, resides the power to select that which is for the best interest of the species.

(*e*) The strength with which sex is established in different species of animals is variable. Moreover it is a fact of common observation that all cell elements vary widely in different animals. We should not be surprised to find, then, that a determinant would exhibit marked varieties of form which might even be carried to the extreme of its entire suppression as a definite element. Incomplete as are the observations upon the behavior

[1] It is suggestive that in all those cases where there appears to be no cross division of the chromosomes in maturation, nothing like the accessory chromosome has been noted. This would seem to be some indication that there might be two types of division.

of the accessory chromosome in various species, enough evidence is forthcoming to show wide departures from anything that might be considered a typical form. And here it is that it may be possible to secure more or less definite information with regard to the meaning of the accessory chromosome. If a large number of observations show variations that parallel well-marked instances of unusual sex characters, then greatly increased probability will attach to the theory I have advanced.

(*f*) Concerning the bearing of parthenogenesis upon the problem of sex determination, we know little. In eggs, no structure comparable to the accessory chromosome has yet been observed and the presence of any such element is extremely improbable. But it is known that different sexes come from parthenogenetic eggs, and in the familiar example of the aphides, these are produced in strict response to environmental demands.

Parthenogenesis, however, is regarded as a degenerate method of sexual reproduction in which polar bodies perform the function of the spermatozoa. Sex might, therefore, be determined by the particular polar body that restored the needed amount of chromatin to the egg, for these, like the spermatozoa, would be of two kinds where a reduction division took place in the process of maturation. These facts would indicate an element of truth in Minot's view regarding the meaning of the polar bodies. In respect to this matter, however, we have only theory to guide us and must wait for more thorough study of the question.

The suggested hypothesis affords a reasonable basis for a number of theories that have been advanced and supported upon empirical data. Among these are Thury's and Düsing's on the time of fertilization; the ones relating to the nutrition of the parents and embryo; and possibly others in which age or "comparative vigor" is assigned as the influential factor.

In general, I would point out, my theory confirms these by showing that the condition of the ovum determines which sort of spermatozoon shall be allowed entrance into the egg substance. In this we see an extension, to its ultimate limit, of the well-known *rôle* of selection on the part of the female organism. The ovum is thus placed in a delicate adjustment with regard to surrounding conditions and reacts in such a way as to best sub-

serve the interest of the species. To it come the two forms of spermatozoa from which selection is made in response to environmental necessities. Adverse conditions demand a preponderance of males, unusually favorable circumstances induce an excess of females, while normal environments apportion an approximately equal representation to each of the sexes.

Those theories regarding sex determination which contain any element of truth within them will be found dependent upon this principle. It is expressed by Geddes and Thompson in these words: "But the general conclusion is tolerably secure—that in the determination of the sex, influences inducing katabolism tend to result in production of males, as those favoring anabolism similarly increase the probability of females." The authors just cited clearly recognize that we must consider the sexual elements in the light of their elemental structure and function when the final explanation of sex is sought. They say: "That the final physiological explanation is, and must be, in terms of protoplasmic metabolism, we must again, however, remind the reader."

The *rôle* that I have suggested for the accessory chromosome in no way changes the ordinary conception of the part played in sex determination by the various observed factors, but it does offer some tangible means by which to correlate these and to fix the nature of their participation.

The conception of two forms of sexual elements which would be operative in the determination of sex is not new. It has been assumed on purely theoretical grounds that there are two kinds of ova, one of which, in the event of fertilization develops into a male organism while the other under similar conditions gives rise to a female. This theory is dismissed by Geddes and Thompson on the ground that the two forms of ova have never been observed and for the further reason that later influences might possibly change the earlier tendency.

The latter objection would prove fatal to any theory which located the determination of sex in a structural difference of the germinal elements. I do not consider this position well taken for reasons that I will give later. The more serious objection lies in the fact that, so far as observation has gone, all eggs of a species are practically alike. It is also to be depreciated because

of the fact that it reverses the ordinary relations of the elements and removes the power of choice from the female.

We have in the case of the spermatozoa, however, the observed fact that there are two essentially different forms and that they are present in equal proportions. No other feature, save sex, separates the resulting offspring into two approximately equal groups. By exclusion then, it would seem that the determination of this difference is reposed in the male element.

There are, I am aware, certain observations upon the determination of sex with which my hypothesis does not seem to agree. Some of these I should like to mention in order to suggest possible explanations or reasons for regarding them within the limit of error set by our present knowledge of the subject. These objections may be suggested by the following questions:

Is sex potentiality—by which I mean the tendency of the species to perpetuate itself in individuals of two sexes of approximately equal numbers—a constant and uniform factor prevailing throughout all classes of animals? Is sex determined at the time of fertilization; if so, is such determination absolute, or may it be changed by varying conditions? Under the unusual circumstance of parthenogenesis, will it be possible to reconcile a theory which postulates the presence of a determinant in the male element with the fact of the entire absence of this element in unisexual reproduction?

An answer to the first question is not difficult. It is a matter of common observation that all animals are not alike in their methods of reproduction. In the insects, for instance, it is known that certain forms invariably produce young after the sexual method and that parthenogenesis never occurs; in others, parthenogenesis is the common method and sexual union of male and female only an infrequent occurrence; while in yet others, one sex is produced by fertilized eggs and the other sex from those unfertilized.

The logical conclusion to be drawn from these facts is that sex, *per se*, is not an unchangeable attribute of organisms but is an adaptation of the species to secure the most favorable conditions for its perpetuation. Given favorable conditions of environment and aphides will reproduce indefinitely with only one sex as a repre-

sentative of the species. Adverse conditions, on the contrary, cause the appearance of the male form which then shares with the female the representation of its kind

Again, some species are influenced by favorable external conditions to such an extent as to cause, not the entire suppression of the male form, but only its subordination in numbers. Finally, there are forms where the numerical proportion of the sexes is preserved with only slight variation even under great extremes in environment. The first case we would consider as an example of a weak, the second as one of moderate, and the last as one of strong, sex potentiality.

Let it be granted, then, that the demand for sexual representation is not equally strong in all species. It follows that we may expect to find corresponding variations in the method by which sex is determined. Such forms as exhibit a ready response to environmental conditions will certainly be more easily influenced even at a late stage of development than would the more stable forms at the beginning.

On this account, an answer to the second question could not be a simple one. It is very probable that, in certain species, sex is determined at the time of fertilization and can not be altered by any later influences. Conversely, it has been experimentally proved that the proportion of sexes may be materially altered by changed nutritive conditions operating upon larval forms, or may possibly be changed several times in the same individuals. But because Yung raised the proportion of females from a normal one of about 56% to the unusual one of 92% in the sexually unstable tadpoles of the frog, it does not follow that in all forms sex is such a variable factor. It is simply an evidence that sex is not a fixed attribute of organisms and that in this particular case it is extremely unsettled. By no means can it be taken as an argument that sex may not be established in the act of fertilization.

In refuting this view, moreover, we are not forced to rely entirely upon negative inferences. In the case of the honey-bee, it has long been known that sex depends solely upon the matter of fertilization. From the impregnated eggs come the females, queens or workers as circumstances dictate; from the unim-

pregnated eggs always males. This fact Dzierzon demonstrated by observation in 1853, and the absence of spermatozoa from the eggs which develop into drones has very recently been proven in the laboratory of Weismann by the use of modern cytological methods.

A further proof, although inferential, is that afforded by "true" twins, in which case it appears that the sex of the two individuals is always the same. If sex were established at the time of fertilization of the ovum, then sex would be shared along with the other qualities possessed by the normal individual that would have developed from the ovum under ordinary conditions. In case sex were not established at the time of impregnation, it would be natural to expect the two sexes to be occasionally represented in one birth because of the inequality of nutrition in the embryos or for other reasons.

Sex, then, *is* determined sometimes by the act of fertilization and can not be subsequently altered. But between this extreme and the other of marked instability there may be found all degrees of response to environment. It must accordingly be granted that there is no hard-and-fast rule about the determination of sex, but that specific conditions have to be taken into account in each case. The objection that Geddes and Thompson raise against the possibility of two forms of eggs, viz., that it is a useless adaptation on account of the fact that subsequent conditions may determine sex in some cases, is not a valid one in general. Such *may* be the case in some instances, but such *is* not the case in others.

Finally, with respect to the evidence to be derived from parthenogenesis, it should be remembered that we are here dealing with a practical suppression of sexuality and it is to be expected that extensive modifications of the ordinary process will follow. If the egg takes upon itself all the functions commonly exercised by it in conjunction with the spermatozoön, it must be that the determination of sex is included. This, in some instances, is a final choice on the part of the ovum and ever afterward one sex only is produced by it; again, however, it maintains a responsive attitude toward environments and gives rise to the sex most needed by the species. It is to be hoped that the

very promising field opened up by the work upon artificial parthenogenesis will throw much light upon these vexed problems.

LABORATORY OF ZOÖLOGY AND HISTOLOGY,
UNIVERSITY OF KANSAS, January 1, 1901.

SUPPLEMENT.

During the period of a year and a half that has elapsed since the completion of the foregoing article, a number of important changes in, and additions to, our knowledge of the accessory chromosome have been made. These are noted in another paper, "The Spermatocyte Divisions of the Locustidæ," soon to be published, so that extended reference to them will not be given here. For the sake of completeness, however, I deem it proper to make brief mention of such as affect the main points of this contribution.

First, I may observe that the exact character of the unequal division of the accessory chromosome in the spermatocytes of the Orthoptera has been established. As may be noted in ¶ 42, I could not determine the behavior of this element in *Xiphidium* with certainty. From its absence in large numbers of spermatids, though, I was inclined to support Henking's view that it remained undivided in one of the spermatocytes. In other genera of Locustidæ, *Orchesticus*, *Anabrus*, *Microcentrum*, and *Scudderia*, I have since been able to demonstrate with certainty that the accessory chromosome divides but once in the spermatocytes. Here, unlike *Pyrrhocoris*, the second spermatocyte mitosis witnesses the separation of the chromatids of the accessory chromosome. The end result is, nevertheless, the same in each case.

Further confirmation of the fact may be found in the work of R. de Sinéty upon other species of Orthoptera, in which he records exactly similar processes. I feel safe in stating it as established therefore, that in the Orthoptera—and in the Hemiptera—the accessory chromosome is normally present in exactly one half the spermatozoa.

With regard to the general distribution of the accessory chromosome, I may state that a student in this laboratory, Mr. M. W. Blackman, has been able to demonstrate its presence in the Myriapoda where it evinces, in connection with all the other cell

elements, a tendency to depart from typical appearances. Montgomery has investigated the spermatogenesis of *Peripatus* and declares that the "chromatin nucleolus" is not present in that form. His evidence, however, I regard as not convincing in this respect.

Credit is assigned Montgomery (¶ 24) for the discovery that the accessory chromosome is merely a spermatogonial chromosome that comes over unchanged into the spermatocytes. This must now be withdrawn, for, at present, he accepts the views of Paulmier that it is formed by synapsis from differentiated elements of the spermatogonia. That it *is*, on the contrary, the same in both cell generations, at least in the Orthoptera, is shown by the work of Sutton (¶ ¶ 49, 52).

Perhaps the most important advance in our knowledge of the accessory chromosome pertains to its relation to the other chromosomes of the cell. During the early investigations upon it, the tendency was to consider it widely removed from the type chromosome, but we are now beginning to perceive that practically its only divergence consists in its isolation. This feature is most pronounced in the prophase of the first spermatocyte where the element bears some little resemblance to a nucleolus—enough, in fact, to have induced several investigators to so call it. But, as has already been shown, this is merely superficial, and later researches upon the Locustid cells have brought to light the fact that the accessory chromosome forms a close spireme of its own, and so parallels the activities of the ordinary chromosomes at the point where it seemed most to diverge. We may therefore regard the accessory chromosome as practically normal in its behavior throughout the different cell generations of the testis up to the point where it is thrown into prominence by the unusual action of the remaining chromosomes during the pseudo-reduction. It may well be, as previously suggested, that the distinction bestowed upon the accessory chromosome at this time is due to its fidelity to the type form of division which, at this point, is abandoned by its fellows.

Regarding the theory of its function advanced in this paper, I can say only that it has, if anything, been strengthened by later researches, and more nearly explains the phenomena involved than any other that has been conceived.

BIBLIOGRAPHY.

Carnoy.

'95 La cytodiérèse chez les Arthropodes. La Cellule, Vol. 1.

Cholodkovsky, N.

'94 Zur Frage über die Anfangsstadien der Spermatogenese bei den Insecten. Zool. Anz., Vol. 17.

Cheshire, F. R.

'86 Bees and Bee-keeping.

Griffin, B. B.

'99 Studies on the Maturation, Fertilization, and Cleavage of Thalassema and Zirphæa. Jr. Morph., Vol. 15.

Geddes and Thompson.

'95 The Evolution of Sex.

Häcker, Valetin.

'97 Ueber weitere Uebereinstimmungen zwischen den Fortpflanzungsvorgängen der Tiere und Pflanzen. Biol. Cent., Vol. 17.

Henking, H.

'90 Untersuchungen über die ersten Entwickelungsvorgänge in den Eiern der Insecten. 2. Ueber Spermatogenese und deren Beziehung zur Eientwickelung bei Pyrrhocoris apterus M. Z. wiss. Zool., Vol. 51.

Henking, H.

'92 Idem 3. Specielles und Allgemeines, ibid., Vol. 54.

Ischikawa.

'91 Studies of Reproductive Elements. Spermatogenesis, Ovogenesis and Fertilization in Diaptomus sp. J. Coll. Sc. Imp. Univ. Japan, Vol. 5.

McClung, C. E.

'99 A Peculiar Nuclear Element in the Male Reproductive Cells of Insects. Zool. Bull., Vol. 2.

McClung, C. E.

'00 The Spermatocyte Divisions of the Acrididæ. Kan. Univ. Quart., Vol. 9, No. 1.

Montgomery, T. H., Jr.

'98 The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. Zool. Jahrb., Vol. 12.

Montgomery, T. H., Jr.

'98 Chromatin Reduction in the Hemiptera : a Correction. Zool. Anz., Vol. 22.

Moore, J. E. S.

'95 On the Structural Changes in the Reproductive Cells during the Spermatogenesis of Elasmobranchs. Quart. J. Micr. Sc., Vol. 38.

Paulmier, F. C.

'93 Chromatin Reduction in the Hemiptera. Anat. Anz., Vol. 14.

Paulmier, F. C.

'99 The Spermatogenesis of Anasa tristis. Jr. Morph., Vol. 15.

Platner, G.

'86 Die Karyokinese bei den Lepidopteren als Grundlage für eine Theorie der Zelltheilung. Internat. Monatsschr. Anat. Histol., Vol. 3.

Rath, O. vom.

'92 Ueber die Reduction der chromatischen Elemente in der Samenbildung von Gryllotalpa. Ber. naturf. Ges. Freiburg, Vol. 6.

Rath, O. vom.
'92 Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris Latr. Arch. mikr. Anat., Vol. 40.
Rath, O. vom.
'95 Neue Beiträge zur Frage der Chromatinreduction in der Samen und Eireife. Ibid., Vol. 4.
Sutton, W. S.
'00 The Spermatogonial Divisions of Brachystola magna. Kan. Univ. Quart., Vol. 9, No. 2.
Toyama, K.
'94 On the Spermatogenesis of the Silkworm. Bull. Coll. Agric. Imp. Univ. Japan, Vol. 2.
v. la Valette St. George.
'97 Zur Samen- und Eibildung beim Seidenspinner (Bombyx mori). Arch. mikr. Anat., Vol. 50.
Wagner, J.
'96 Beiträge zur Kenntniss der Spermatogenese bei den Spinnen. Arb. Nat. Ges. St. Petersburg, Vol. 26.
Wallace, Louise B.
'00 The Accessory Chromosome in the Spider. Anat. Anz., Bd. 18, Nos. 13 and 14.
Weismann, August.
'00 Ueber die Parthenogenese der Bienen. Anat. Anz., Bd. 19, Nos. 20 and 21,
Wilcox, E. V.
'95 Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. Bull. Mus. Comp. Zool. Harvard Univ., Vol. 27.
Wilcox, E. V.
'96 Further Studies on the Spermatogenesis of Caloptenus femur-rubrum. Ibid., Vol. 29.
Wilcox, E. V.
'97 Chromatic Tetrads. Anat. Anz., Vol. 14.
Wilson, E. B.
'00 The Cell in Development and Inheritance. New York. 2d Ed.

THE METALLIC COLORS OF FEATHERS FROM THE NECK OF THE DOMESTIC PIGEON.

R. M. STRONG.

The so-called metallic colors and iridescent effects exhibited by many feathers have been variously explained by different writers. In general they are recognized as diffraction phenomena peculiar to the barbules.

If one observes the neck of a gray domestic pigeon by reflected sunlight, the feathers on the sides will appear bright metallic-green when the angles of incidence and reflection are small. But when the angles are large, the same region has a purplish appearance. Under other conditions a dull brown color prevails. A single feather gives metallic colors only from the distal exposed portion.

The hypotheses based on the supposed presence of striæ or ridges, supported by Haecker ('90) and others are seen at once to be inapplicable to this case when one finds that the feather may be rotated through a whole circle with essentially the same color effects for given angles even from individual barbules. Furthermore, a careful microscopic study of the barbule surface shows that irregularities such as striæ, ridges, pits, knob-like elevations, etc., are not frequent enough when sufficiently small to produce grating effects, and in fact are not normal occurrences.

On comparing barbules giving metallic colors with barbules from the same barb in a region of non-metallic colors, striking modifications are found. The barbules from the region where metallic colors do not appear are of the typical form, with a proximal flattened region, a longer attenuated portion, and well-developed barbicels on the distal barbules. They present their dorsal margins upward in a dorsal view. There is a comparatively sparse pigmentation with the typical rod-shaped granules of melanin found in feathers.

In the region of metallic colors, one finds the proximal barbules essentially like the distal barbules and flattened throughout their extent. The attenuated portions characteristic of typical

barbules are absent as are also the barbicels. There is a very heavy dark pigmentation of the dorsal halves of the barbules and they are arranged so that they presènt their flattened surfaces upward in a dorsal view. The dorsal margins rest upon the median regions of the next more distal barbules and the ventral margins are thereby hidden from view. The arrangement suggests, somewhat, two rows of shingles overlapping each other on either side of a median axis.

On sectioning these barbules, I find an outer transparent layer less than one micron in thickness, which encloses cell cavities more or less closely packed with *spherical* granules of melanin pigment less than one micron in diameter. The greater part of this pigment is in the dorsal half of the barbule, the part visible in a dorsal view of the feather.

For several months I held strongly to the view that the thin transparent layer produced the well-known interference colors of thin plates, as was supposed by Altum ('54, '54a) and Brücke ('61). The exceedingly uniform size and unusual shape of these pigment granules seemed too significant, however, to warrant an unqualified acceptance of the thin-plate hypothesis. Usually, melanin granules become more or less generally fused together into irregular masses or are imbedded in the horn substance of the feather. In the barbules which give metallic colors, however, the spherical granules retain their individual form. Cross sections frequently show them scattered about outside the barbule section and they seem to have been simply packed in the cell cavities of the barbules with little or no cementing substance to hold them together.

In the feather germ, I find typical pigment cells or chromatophores which produce typical rod-shaped granules. But after these granules have passed into the cells composing the fundaments of the barbules which are to have metallic colors, they are transformed into spherical granules.

By a fortunate manipulation of apparatus, recently, I was able to view barbules by strong reflected sunlight while using a Leitz No. 7 objective. A beautiful pattern of gleaming spheres was presented to my eye. Each pigment granule appeared to be diffracting light and all the colors of the spectrum were visible in the field. On the broad surface of the barbule, where the

angles of incidence were small, the predominant colors were yellow and green, especially green. On the margins where the angles of incidence were great, the prevailing colors were reds and purples.

The colors of the feathers described, when observed without a microscope, are very apparently mixed colors. The greenish effects are produced when light strikes the broad surfaces of the barbules and is reflected with a small angle of reflection. The reds appear only when light falls with a large angle of incidence on the pigment granules of a margin or elevated portion of a barbule.

We seem to have a clear case of Newton's rings where each pigment granule comes in contact with the outer transparent layer.

This is a preliminary statement of my results. Another paper describing them in greater detail with the aid of figures is in process of publication.

APRIL 1, 1902.

BIBLIOGRAPHY.

Altum, B.

'54. Ueber den Bau der Federn als Grund ihrer Färbung. Journ. für Ornith., Bd. 2, pp. xix–xxxv.

Altum, B.

'54a. Ueber die Farben der Vogelfedern im Allgemein, über das Schillern insbesondere. Naumannia, Jahrg. 1854, pp. 293–304.

Brücke, E.

'61. Über den Metallglanz. Sitzungsberichte d. Kais. Akad. in Wien. Math. Naturw. Classe, Bd. 43, Abth. 2, pp. 177–192.

Haecker, V.

'90. Ueber die Farben der Vogelfedern. Arch. f. mik. Anat., Bd. 35, pp. 68–87, Taf. 4.

STUDIES IN EARTHWORM CHLORAGOGUE.

WM. J. RICE.

The dearth of knowledge concerning the physiological significance of earthworm chloragogue and the opportunity offered for the study of the subject, are the main reasons for the work here presented, which has been carried on under the direction of Professor James G. Needham.

I have found only one article having an important bearing on the subject of this paper, one by Thomas Schaeppi,[1] which was found after this research was practically completed. It may be stated that in several respects a marked similarity exists between the chloragogue of *Ophelia radiata* and that of the earthworm, especially as to color, strong resistance to acid and alkaline reagents and the probability that the chloragogue is excretory in function rather than secretory.

Lumbricus herculeus has served for the most part as material for study. As much as was possible of the work was done with freshly collected material. But when, during the winter months, fresh material was not available, worms were used which had been collected in the late fall and, to insure a thrifty condition, preserved alive for winter use under favorable conditions of temperature and moisture. The worms were found to thrive best in temperature varying from 40° to 60° F. Extremes of heat were quickly fatal. It was found necessary to exercise care in the amount of moisture furnished, as too much moisture was as hurtful as too little. The results obtained from material properly preserved and from freshly collected material were essentially the same.

The following points are considered: I. Origin and growth of chloragogue; II. Distribution; III. Structure; IV. Function and V. Elimination.

I. *Origin and Growth.*—To learn whether the chloragogue, as is generally supposed, is a modification of the peritoneal epithelium, a series of worms was prepared beginning with a young

[1] "Das Chloragogen von Ophelia radiata" Jenaische Zeitschrift für Naturwissenschaft, 1893, XXVIII., Neue Folge XXI.

worm 5 mm. long and continuing through increasing sizes till a stage was reached in which the chloragogue cells appeared fully developed. In the smallest worms the peritoneal layer, the chloragogue that is to be, is always apparent and a similarity is evident between it and the peritoneal layer elsewhere, both in structure and in reaction to staining fluids. In the smallest worms the chloragogue appears as a simple layer as yet undifferentiated into the club-shaped cells of the adult form. In larger specimens of the series a gradual development into the typical chloragogue cells can be traced. As the cells become older they increase in length, the characteristic granules appear and they become less and less responsive to stains, until in the fully developed adult condition they are practically proof, excepting their nuclei, against every stain to which they were subjected. The nuclei readily respond to stain.

Upon adult worms the following experiment was performed to determine whether there was any renewal of chloragogue when it is artificially removed. With a sharp razor an incision was made through the outer body wall into the cœlomic cavity, exposing the alimentary wall. With a scalpel the chloragogue was then removed from the alimentary wall as completely as possible and the worm afterward kept under favorable conditions of temperature, moisture, etc., in order to insure a condition of thrift. The result of this experiment was that although the wound made in the body wall in order to remove the chloragogue began to heal well the second day after it was made, no chloragogue regeneration was observed in specimens sectioned at various intervals thereafter.

II. *Distribution.*—In the adult worm the chloragogue is found to be distributed as follows: beginning at the posterior end of the œsophagus it extends dorsally, attached in abundance to either side of the dorsal blood vessel as far back as the thirtieth segment (counting from the rear). It is interesting to note that the chloragogue attached to the outside of the alimentary canal *practically* ends in the region where the typhlosole ends: from the thirtieth segment to the anus it becomes less and less abundant, finally disappearing about the seventh somite. In the typhlosole it is also abundant. Somewhat less abundant than

on either side of the dorsal blood vessel, the chloragogue is found surrounding the origin of the aortic arches dorsally, and covering the dorso-intestinal blood vessels, an important fact, inasmuch as the blood gathers from the alimentary wall into the dorso-intestinal blood vessels and through them flows into the dorsal blood vessel. This condition suggests that the chloragogue may with good reason be considered as having to do with food elaboration. The chloragogue is found less abundant still on the outer surface of the alimentary wall, arranged in irregular circular bands. It is also found laterally and ventrally but not as abundantly as on the dorsal aspect. It is entirely absent from the ventral blood vessels, in marked contrast to its abundance on the dorsal vessels. In passing, reference may be made to the fact that within the dorsal blood vessel as seen in cross section a tissue is found, which in appearance, is much like the chloragogue. The largest individual chloragogue cells are found on either side of the dorsal vessel as it crosses the crop and gizzard. The walls of the crop and gizzard are free from chloragogue.

III. *Structure.*—The typical adult chloragogue cell is club-shaped, having at its narrow base a nucleus of medium size which readily stains, while filling the remaining cell space are innumerable minute yellow granules which compose the greater part of the cell and give to it its characteristic color, which varies from a brownish-yellow to a yellowish-green. These chloragogue granules are apparently lifeless. No stain has been found to affect them, the strongest acids like nitric acid and hydrochloric acid affect them but slowly. Likewise strong alkalies like potassium hydroxide have only a very slow effect in disintegrating them. Neither does feeding or starving a worm, as will be shown later, have any apparent effect upon the chloragogue. In the very young worm, however, the entire chloragogue cell is more or less responsive to stains, and the younger the chloragogue the more striking is its likeness to the peritoneal epithelial layer of which it is generally supposed to be a modification.

IV. *Function.*—One of the first attempts to determine the function of the chloragogue was through special feeding. A long series of negative results was obtained. The difficulty was not

found in the worms refusing to eat, because a few hours (over night) sufficed for a worm to gorge itself with whatever substance was placed before it even though it were no more than common white blotting paper. The worm, when the proper amount of moisture and the right limits of temperature were provided, thrived as well on fuchsin-stained earth ; on milk-saturated earth;[1] on finely divided meat scraps ; on moist bread ; on vegetables such as finely chopped leaves of cabbage, lettuce, etc., as it did on its customary earth diet. But no effect due to this varied feeding was observed on the chloragogue. This being so it was determined to try and affect the chloragogue through starving. A number of large thrifty worms were deprived of both food and water for as long a time as they could be kept alive, which was three days. In this time they lost 44 per cent. in weight, but a microscopical study in cross section of a worm thus treated, while it showed a great shrinkage in the cells of other tissues, did not show the chloragogue as having undergone any essential change. It cannot here be said that the function of the chloragogue has been definitely learned ; but the following inferences in the light of the foregoing and what is to follow seem justified. Finding the chloragogue in such close proximity to that part of the blood system which transports the newly absorbed food (see Fig. 1)—dorsal blood vessel, dorso-intestinal blood vessels and aortic arches—and within the typhlosole, and ceasing to be abundant on the outside of the alimentary wall at the point where the typhlosole ends within the alimentary canal, would indicate that the chloragogue had an important relation to the elaboration of food. But the lifeless condition of the adult chloragogue and its apparently living condition in the very young worm, indicate that possibly the function of the chloragogue is performed in the early development of the worm, and that in the adult it has become functionless, although in the

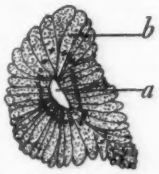


FIG. 1. Showing the characteristic abundance of chloragogue surrounding the dorso-intestinal blood vessels. *a*, dorso-intestinal blood vessel with (*b*) chloragogue cells surrounding it.

[1] Here it may be added that in histological preparations of milk-fed worms, treated with osmic acid, the characteristic black-colored fat globules could be distinguished in the epithelial cells of the alimentary wall but no trace of them was found in the chloragogue cells.

light of what follows the inference seems justified that it may have an excretory function.

V. *Elimination.*—Chloragogue granules are found free in the cœlomic fluid. They are found imbedded in leucocyte bodies. They are found composing the greater part of large waste masses,

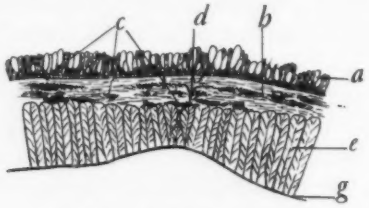


FIG. 2. Transverse view of median dorsal section of outer body wall showing characteristic elimination of minute waste masses of chloragogue granules. *a*, epithelial cells (hypodermis); *b*, circular layer of muscles; *c*, minute masses of waste chloragogue granules making their way to the outside dorsally. In Fig. 3 the masses are large but less numerous; *d*, nerve; *e*, longitudinal muscle layer; *g*, peritoneal epithelium of which the chloragogue is a modification.

in some instances filling the entire cœlomic cavity in the posterior region of the body, between numbers of the dissepiments. Lastly they are found in the muscular tissue of the outer body wall, dorsally and ventrally (see Figs. 2 and 3): on the dorsal

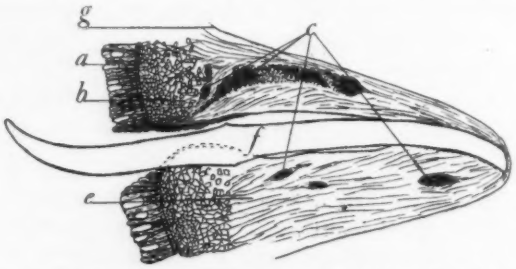


FIG. 3. Longitudinal view of portion of the outer body wall at the level of the ventral row of setæ showing waste elimination ventrally, of waste chloragogue granules in considerable mass. Letters signify same as in Fig. 2 with addition of *f*, seta.

aspect, for the most part as diffuse granular masses distributed throughout the circular muscular layer; on the ventral aspect in more compact masses in the vicinity of the setæ. They appear to be thus making their way to the outside. That there is

in the earthworm such a crude method of waste elimination is further supported by the results of an experiment performed by a fellow student at Lake Forest College, Mr. John J. Jackson. His experiment was undertaken to learn how the earthworm eliminates a foreign substance injected into the cœlomic cavity. Lamp-black was the substance used on this occasion. Histological preparations of material thus treated showed the lamp-black evidently making its way through the tissues of the outer body wall to the outside in the manner described for the waste chloragogue granules. That the letter are thus directly extruded any one may easily demonstrate by the following experiment: A thrifty worm thoroughly washed is placed under a bell-jar and subjected to the influence of ether vapor. The mucus which the worm casts off as a result of this ether vapor irritation, on microscopical examination is seen to contain an abundance of chloragogue granules which are enveloped or embedded in a transparent substance—possibly the contents of a mucous or a leucocyte cell that soon bursts, allowing the granules to scatter in various directions.

In the waste masses, for the most part composed of chloragogue granules, found in the anal region are large numbers of setæ, in one adult worm as many as six hundred and twenty-five (counted in part and estimated) were present, varying in size from small undeveloped to large fully developed ones. The size of the setæ depends somewhat on the size of the worm—the fully developed setæ naturally not being found in a very young worm while in an adult worm both very small and fully developed setæ are often present. Also in many of these same waste masses are found nematodes—*Anguilula lumbricci*, kindly determined for me by Dr. W. M. Woodworth. An interesting question arises in regard to the presence of these nematodes embedded in the waste masses in the posterior region of the body because of the following conditions. While some waste masses are entirely lacking in nematodes, such masses being of a dark brown color, other waste masses again are found which vary from a brown to an almost white color—depending on the number of nematodes present. If they are many, in which case the granules of chloragogue as a rule are scarce, the masses are white. The question

that has arisen from this condition of things is whether there may not be a symbiotic relations between the earthworm and these supposed parasites. Are these nematodes instrumental in consuming the chloragogue waste which the worm by other means is unable to eliminate from its body?

A detailed study of these waste masses both in preserved (for winter use) and freshly collected material gave these results: The average number of setæ (counted in part and estimated) present in the waste masses of each of the seventeen worms examined was two hundred and forty-eight. The nematodes in the dark-colored waste masses were as a rule few—sometimes entirely absent—and, when present, in a quiescent state, often enveloped by a sort of cyst. In these dark-colored masses the chloragogue granules far exceeded the nematodes in abundance.

In the light-colored masses, on the other hand, the nematodes were far in excess of the chloragogue granules, and on pressing the cover-slip many of the nematodes, having within their bodies granules apparently identical as to size and color with those of the surrounding waste chloragogue granules, could be excited to move freely and by so doing clearly showed themselves to be unencumbered by any cyst. Their appearance was suggestive of a newly moulted insect. This supposition of a symbiotic relation is then supported to the extent that within the nematodes have been found granules identical as to size and color with chloragogue granules; and further by the fact that where the nematodes are in excess and in an active condition the chloragogue granules as a rule are far less abundant than the nematodes. It should be stated, however, that the nematodes at no time have been seen in the act of consuming the chloragogue granules, though frequently one has patiently sought to observe this. Still the facts seem to justify the suggestion of a possible symbiotic relation existing between these nematodes and the earthworms within which they live.